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**ADRENAL CORTICAL STUDIES
IN
CORONARY ARTERY
DISEASE**

-RAMSAY-

FOR REFERENCE

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THE UNIVERSITY OF ALBERTA

ADRENAL CORTICAL STUDIES IN CORONARY ARTERY DISEASE

A DISSERTATION

SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES
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by

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PREFACE

The following thesis consists of a report of an attempt to elucidate the relationship of the serum cholesterol level of the human to the activity of the adrenal cortex. The reasons for undertaking this project stemmed from a previous clinical experiment of Conn and his co-workers which purported to prove that the serum cholesterol level was controlled by the adrenal cortex. It was decided, therefore, to attempt to confirm Conn's observations and, if confirmation was possible, to assess the value of the serum cholesterol level as an index of adrenal cortical activity. In addition it was hoped the serum cholesterol level and the eosinophil count might prove aids in prognosticating the outcome of severe physical stress. This hope arose from Selye's work suggesting that death during severe episodes of disease might be the result of adrenal cortical exhaustion.

In order to achieve the aims outlined above, it was decided that a detailed study of the clinical condition, the cortin and 17-ketosteroid excretion, and the eosinophil levels would provide the necessary information. No attempt was made to set up a survey planned from the statistical point of view because it was felt that the day-to-day observations of such a comprehensive study would demonstrate clearly any important correlations that existed. Although some suggestions of the relationship of the serum cholesterol level to the degree of adrenal cortical activity were obtained, the objects of the experiment were somewhat vitiated by the fact that the indices of adrenal cortical function used in this experiment failed to agree with each other. Insofar as it was possible to tell, however, no direct relationship

exists between adrenal cortical activity and the total serum cholesterol level.

The introduction gives a short explanation of the value of the eosinophil count, and the cortin and 17-ketosteroid excretion as indices of adrenal cortical function. An account is given of the various experimental findings which led up to Conn's study. Since this present study attempts to demonstrate the relationship of adrenal cortical secretory output and cholesterol during stress, the previous observations on the effect of stress of the serum cholesterol level are given with alternate explanations for the changes in cholesterol level during stress. In order to elucidate the possible prognostic value of cholesterol, Selye's theories are presented.

In conclusion I wish to express my appreciation to all the people whose cooperation made this work possible - to the John and Mary R. Markle Foundation for their unselfish support, to Dr. D. R. Wilson, my patient and esteemed mentor, to Dr. R. E. Bell and Dr. H. V. Rice for their invaluable advice and assistance and last, but not least, to the medical and laboratory staff of the University of Alberta Hospital for a great deal of technical aid.

F. W. Ramsay.

TABLE OF CONTENTS

	<u>Page</u>
<u>INTRODUCTION:</u>	1
A. Adrenal Cortical Function	1
I. The Nature of Adrenal Cortical Function	1
II. Measurement of Adrenal Cortical Secretion	3
(a) Changes in the sudanophilic material and cholesterol content of the adrenal cortex	3
(b) Changes in the level of circulating eosinophils	4
(c) Urinary corticoid excretion	5
(d) Urinary 17-Ketosteroid excretion	6
III. Response of the Pituitary-Adrenal Cortical System to Stress	7
(a) Morphological and chemical evidences of adrenal cortical response to stress	7
(b) Exhaustion of the adrenal cortex	9
(c) Exhaustion of the adenohypophysis	10
(d) Adaptation to stress	11
B. Relationship of the Adrenal Cortex and the Serum Cholesterol Level..	15
I. Effects of Stress on the Plasma Cholesterol Level	15
II. Conn's Theory	19
III. Decreased Synthesis	24
<u>METHOD:</u>	29
A. Rationale	29
B. Clinical Material	31
C. Hospitalization	32
D. Clinical Observations	32
E. Collection of Samples:	
(a) Blood	32
(b) Urine	33
F. Technical Methods	34
<u>OBSERVATIONS:</u>	34
A. Variability of the Cholesterol Level	34
B. Observations on the Relationship of the Total Serum Cholesterol Level to the Degree of Adrenal Cortical Activity during Stress	36
C. Summary of Observations	43
<u>DISCUSSION:</u>	46
A. The Effect of Stress on the Serum Cholesterol Level	46
B. The Relationship of the Serum Cholesterol Level in Stress to Adrenal Cortical Activity	49
<u>SUMMARY AND CONCLUSIONS:</u>	59
<u>APPENDIX:</u>	

INDEX OF FIGURESOPPOSITE PAGE

1. The structural relationships of cholesterol to the adrenal cortical hormones.....	2
2. Selye's concept of the changes in resistance of an organism to superadded injury during a prolonged period of stress.....	13
3. Alterations in the total blood lipids and blood cholesterol and in the urinary corticoid and 17-ketosteroid output in stress	13
4. Changes in the serum cholesterol levels of 10 patients after a heart attack	35
5. Data on Mr. F.C. #17472	36
6. Data on Mr. A.M. #56542	37
7. Data on Mr. S.S. #105948	38
8. Data on Mrs. M.A. #6091	39
9. Data on Mr. D.B. #107133	39
10. Data on Mr. W.T.L. #94483	40
11. Data on Mr. E.M. #107226	41
12. Data on Mr. Q.M. #72381	42
13. Data on Mrs. M.R. #10065	42
14. Data on Mr. P.S. #7638	43
15. The relationship between the serum cholesterol and the haematocrit level of each of 10 patients following a heart attack	48
16. Continuation of Fig. 15	48

INTRODUCTION

A. Adrenal Cortical Function.

1. The Nature of Adrenal Cortical Secretion.

With the sole exception of the pituitary gland, no gland of internal secretion in the mamalian organism possesses such a multiplicity of functions as the adrenal cortex. All the ramifications of the effects of adrenal cortical secretion have by no means been traced and yet the body of information the subject is formidable. Naturally one of the facets of adrenal secretion which has received much attention is the nature of the adrenal cortical hormones themselves. Between fourteen and twenty-five chemically pure steroid compounds have been isolated from the adrenal cortical glands of animals. The exact number isolated depends upon the extraction process, the earlier chemical methods (1) being harsher and consequently more productive of artifacts than the more recently applied method of paper chromatography (2). Despite their large number, the nature of the adrenal cortical compounds can be fairly easily understood with the aid of certain generalizations, one of the most important to this study being the structural similarity of all of these compounds to each other and to cholesterol. As shown in Fig. 1, all the adrenal cortical steroids possess the large cyclopentanoperhydrophenanthrene nucleus which characterizes the structure of cholesterol.

Of major consequence also is the susceptibility of the hormonal compounds to a simple complementary classification according to function and structure. In all there are three chemical

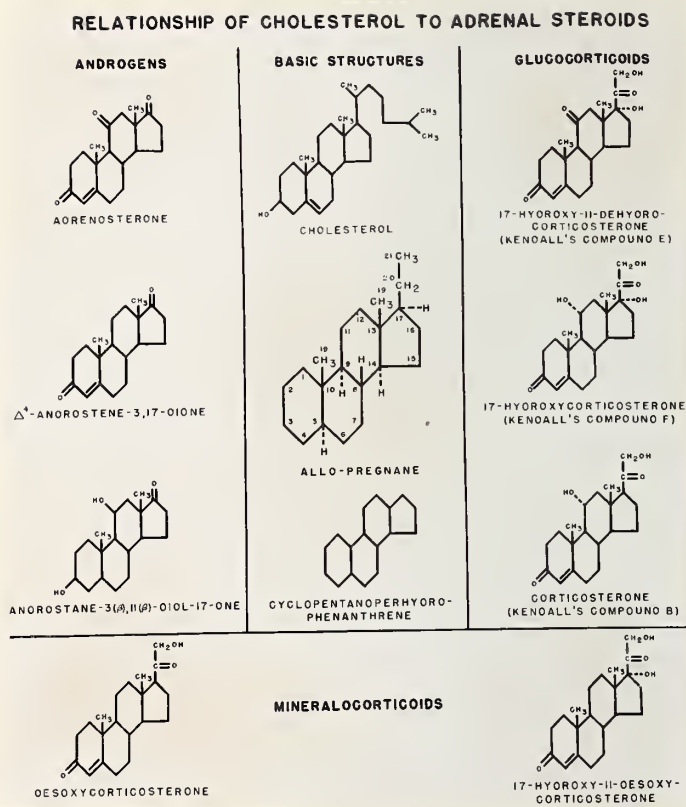


Figure 1. The structural relationship of cholesterol to the adrenal cortical hormones.

groups of adrenal cortical hormones, each possessing distinctive physiological properties.

1. The Desoxy-group (Mineralocorticoids, Na-hormones).

The absence of an oxygen molecule or a hydroxyl group at carbon eleven of the cyclopentanoperhydrophenathrene nucleus (see Fig. 1) distinguishes this group of cortical steroids. The most important member of the group, desoxycorticosterone, actively regulates electrolyte balance in the body fluids and is capable of maintaining life in adrenalectomized animals and patients with Addison's disease.

2. The 11-Oxy- and 11, 17-Oxysteroid Group (Glucocorticoids, S-hormones)

These compounds differ from the preceding group in that all of them have an oxygen or hydroxyl group at carbon eleven and some also have a hydroxyl group at carbon seventeen. The oxygen at carbon eleven makes these steroids effective in influencing the metabolism of carbohydrates, fats and proteins and in controlling the amount of lymphoid tissue and circulating eosinophils in the organism. The best known example of this group is cortisone or 17 hydroxy- 11-dehydrocorticosterone.

3. The Adrenal Androgens (Testoids, N-hormones).

The adrenal androgens characteristically have only a ketone oxygen group attached to carbon seventeen, a side chain on this carbon being detrimental to their physiological properties (3). Their chief metabolic activity consists of their ability to promote nitrogen retention and anabolism or at least to discourage catabolism.

It should be noted that these groups of adrenal cortical

hormones are not air-tight, there being so many structural variants that the groups tend to shade into each other in a qualitative physiological sense.

II. Measurement of Adrenal Cortical Secretion.

One cannot measure directly the output of adrenal cortical hormones from the cortex by any routine method at present. However, measurements can be made which provide an indirect estimate of the adrenal cortical function. One can estimate the changes occurring in the adrenal cortex, in the organism, and in the urinary excretion of steroids as a result of adrenal cortical function. Of the changes in the adrenal cortex itself, the variations in the sudanophilic and cholesterol content of the gland possess some considerable theoretical interest in this study.

(a) Changes in the sudanophilic material and cholesterol content of the adrenal cortex.

The Sudan and Scharlach R stains provide a rough estimate of the amount of cholesterol in the adrenal cortical gland (4, 5) and by the use of these stains histochemical changes have been demonstrated in the cortex. For instance if the gland is relatively inactive, these stains show large amounts of sudanophilic material (mostly cholesterol) in the gland; if the gland is more than normally active, the sudanophilic material undergoes depletion. Changes in the cholesterol content of the gland have been determined directly by chemical determination. Sayers, et al., (4) have shown that the adrenal gland of rats and guinea pigs exhibits a rapid reduction in its cholesterol content during stress. When the stress stops,

the cholesterol immediately begins to increase in quantity (6). Stress (7) or ACTH (8) deplete the adrenal cholesterol stores fifty percent in three hours. The depletion of adrenal cholesterol is accompanied simultaneously by signs of metabolic activity characteristic of adrenal cortical hormones (4). As noted previously, cholesterol is closely related in its molecular structure to all the adrenal cortical hormones. Therefore, because of these facts and because the adrenal cholesterol level remains unchanging at a higher than normal concentration in hypophysectomized rats (7), the adrenal cholesterol has been considered the biochemical predecessor of the adrenal cortical hormones. Indeed Zaffaroni, et al., (10) recently provided strong support for this assumption by showing that as beef adrenal gland perfused in vitro with fluid containing radioactive cholesterol produces radioactive hormones.

(b) Changes in the level of circulating eosinophils.

Of the many changes occurring in the organisms with variations in the amount of adrenal cortical secretion, those occurring in the circulating eosinophil level are of the greatest practical importance in this study. Following the elucidation by Selye (11) of the part played by the eosinophils in the general adaptation syndrome, Hills, et al., (12) observed that the number of circulating eosinophils was under the control of the anterior pituitary-adrenal cortical system. The eosinophil response to stress or ACTH is one of the most useful indices of adrenal cortical function in man. The eosinophil count is a simple, practical procedure.

The lymphocytes also decrease with stress or ACTH administration but the number of lymphocytes, though as simple to count as the eosinophils, is more variable in its response to ACTH and its percentage drop is only half as great as that of the circulating eosinophils (13).

Labor (14), edampsia (15), surgery (14, 15), administration of epinephrin (15, 16), injection of insulin (15, 16), electroconvulsive therapy (17), and other stresses produce eosinopenia in man. Absence of eosinopenia following surgery or the administration of ACTH is indicative of adrenal cortical insufficiency (18).

However, some caution must be used in interpreting the eosinophil count, since it has been shown that eosinopenia can occur in response to infections (19) and large doses of epinephrine (20) in patients with Addison's disease. Possibly these patients may have only suffered a relative adrenal cortical insufficiency, however.

(c) Urinary corticoid excretion.

The chemical determination of changes in the excretion of urinary steroids has been one of the most commonly used clinical methods of estimating the degree of adrenal cortical activity. The urinary steroids generally fall into two groups - the corticoids and the 17-ketosteroids. The corticoids are substances extracted from urine which have some of the properties of the cortical hormones but which, until recently, have not been isolated in pure form and have, therefore, been called corticoids or, collectively "cortin". The corticoid excretion is believed to be derived chiefly from the glucocorticoid and mineralcorticoid output. In the cortin assay

method employed in this study, that of Daughaday, et al., (21) formaldehyde is generated by oxidation of the alpha-ketol or glycol side chains of the urinary corticoids and then is distilled over into water and measured colorimetrically.

Generally there is a good correlation between the urinary corticoid level determined chemically and the clinical evaluation of the adrenal cortical hormone output, despite the fact that the urinary corticoid excretion has been estimated as only a fraction of the cortical hormone output (22). In Addison's disease, however, an appreciable amount of urinary corticoids is still excreted in the urine (23).

(d) Urinary 17-ketosteroids.

Urinary 17-ketosteroids may be estimated by two or three simple and accurate chemical methods, that of Holtorff and Koch (24) being used in this study. Under an optimal environmental state, the adrenal cortex contributes two-thirds of the 17-ketosteroids excreted by the male and all the 17-ketosteroids excreted by the female. The testis provides the other third of the 17-ketosteroid excretion of the male. The urinary 17-ketosteroids are composed partly of steroids which are active androgenically and partly of non-active steroids. They may be the end-products of a normal androgen secretion of the adrenal cortex or they may possibly be the end-products of the degradation of the mineralocorticoids and the glucocorticoids secreted by the adrenal cortex. The adrenal androgens may have a position intermediate in the breakdown of the above mentioned hormones to the 17-ketosteroids. Facts suggesting that 17-ketosteroids are derived from the 21-carbon cortical hormones are the parallel

rise in 17-ketosteroids, and corticoids, which occurs during ACTH administration (13, 25, 26, 27) and the rise in 17-ketosteroid output which accompanies cortisone administration (28). Furthermore, 17-ketosteroids have been isolated which possess an oxygen at carbon eleven, a feature characteristic of the glucocorticoids (29, 30, 31).

According to Sayers, 17-ketosteroids should be rejected as a measure of adrenal cortical activity during stress because of a lack of parallelism between 17-ketosteroid excretion and cortin (or corticoid) output in stressful conditions (32). In stress, Forbes, et al., (33) have shown at the beginning of the stress period there is only a short period of one to three days when the 17-ketosteroid excretion is elevated and that it then usually drops below normal for a much longer period until convalescence is almost complete. Hard physical work (34) and starvation (35) have been found to diminish the 17-ketosteroid output. 17-ketosteroid excretion has also been found to decrease with age, although cortin excretion does not (36). However, 17-ketosteroid excretion decreases markedly in adrenalectomized animals (37) and in patients with Addison's disease (32, 38). Also, during the emotional tension of real or simulated flying, 17-ketosteroids have been found to increase (39). As Mason and Engstrom (40) have pointed out in a review article on 17-ketosteroids, during stress the metabolic disposal of 17-ketosteroids may be changed.

III. Response of the Pituitary-Adrenal Cortical System to Stress.

(a) Morphological and chemical evidences of adrenal cortical response to stress.

Sayers and his group (4) have studied the morphological and

chemical changes in the adrenal cortex in various states of stress produced by a variety of conditions upsetting to the organism. The object of Sayers, et al., was to obtain the most direct type of information possible on the kind of response of the adrenal cortex to varying stimuli. They found that the cortical response was affected by the intensity and duration of the stressful circumstances. As a result of the study Sayers (41) proposed the following scheme as a classification of the types of adrenal response to stress.

Type I. Sudden Temporary Period of Stress. When the alarming stimulus acts for a short period (a few hours), the sudanophilic material, cholesterol, and ascorbic acid in the adrenal cortex become greatly diminished and then are restored to a normal concentration as the animal recovers. Acute non-fatal hemorrhage (7) and intraperitoneal injection of glucose solution (42) are two stresses producing this adrenal cortical response which is reproduced by a single dose of ACTH.

Type II. Very Gradual Change in Internal or External Environment. When a stimulus gradually increases in intensity over a period of a few weeks or months, the adrenal cortex undergoes hyperplastic changes but no change occurs in the concentrations of sudanophilic material, cholesterol and ascorbic acid. Stresses producing the above response are fasting (43, 44), caloric restriction (45), pregnancy (46), and mild chronic infection (47).

Type III. Intense Continuous Stress Ending in Death. In infectious diseases with a fatal outcome (48, 49, 50, 51), to mention one example, the stress produces an almost complete disappearance of sudanophilic material, cholesterol, and ascorbic acid from the adrenal

cortex, accompanied by hyperplastic and hypertrophic changes in the cortical cells.

Type IV. Recovery from a Period of Severe Stress.

After undergoing all the changes described in the Type III response, the cortex immediately begins to accumulate cholesterol upon the abrupt cessation of the stimulus. As a result, the cholesterol content of the adrenal gland first returns to normal and then increases to abnormally high levels. Administration of ACTH for a few days followed by sudden withdrawal will reproduce the above mentioned changes following cessation of the therapy (8).

Type V. Adaptation to Stress.

When a noxious stimulus such as exposure to low, atmospheric pressure (52, 53) or temperature (53) is continuously applied to an animal, the adrenal cortex first becomes depleted of its cholesterol. Then as specific defenses are raised against the stimulus and the effects of the stress on the internal environment decrease, the cholesterol concentration returns to a normal level. It may or may not be stored in excess for a variable period of time after alleviation of the effects of the noxious stimulus.

Sayers emphasizes that this division of adrenal cortical responses is arbitrary and that the responses tend to merge into each other.

(b) Exhaustion of the adrenal cortex.

In the literature much discussion has centred about the possibility that exhaustion of the adrenal cortex might occur with fatal results during severe prolonged stress. Selye has based his

concept of the General Adaptation Syndrome on the premise that such exhaustion of the adrenal cortex does occur (11). He has gone so far as to title the final phase of the General Adaptation Syndrome the exhaustion phase. Thorne, et al., (54) have recommended cortical steroid therapy in patients who develop shock whilst suffering from overwhelming infections. The idea of exhaustion of the adrenal cortex has been fostered by observation that the adrenal cortex following severe stress is depleted of its sudanophilic substance, cholesterol and ascorbic acid. This depletion of these "precursor materials" is thought by some to signify that the adrenal cortex cannot secrete hormones. However, in view of the known ability of the adrenal cortex to synthesize cholesterol, it is quite conceivable that during a severe stress a hyperplastic adrenal cortex might synthesize enough cholesterol and consequently adrenal cortical hormones to satisfy the needs of the organism without being able to produce enough cholesterol to allow a surplus to accumulate. After considering the clinical and laboratory evidence for adrenal cortical exhaustion Sayers has concluded that no satisfactory evidence exists which proves this hypothesis (55).

(c) Exhaustion of the Adenohypophysis.

In the light of the evidence presented by Selye and his co-workers (56, 57, 58, 59, 60) that secretion of ACTH by the adenohypophysis is increased by a high protein intake and decreased by a low protein intake, consideration has been given to the possibility that adrenal cortical activity may be curtailed during severe stress by a block in the synthesis of ACTH. Benura and Howard (61) have produced results contradictory to those of Selye, et al. Burns, et al., (62) has found

that the pituitaries of humans and animals dying under conditions of prolonged stress contain considerable amounts of ACTH. After reviewing the literature on the subject, Sayers (63) arrived at the conclusion that the likelihood is small that the rate of discharge of ACTH is ever a limiting factor in the ability of the adrenal cortex to respond adequately to stress.

(d) Adaptation to Stress.

Adaptation to stress has been defined by Sayers (64) as "that state of an organism characterized by resistance to stress through previous exposure to stress". Specific adaptation is the resistance to a particular stress obtained by an organism through previous exposure to that same stress. Non-specific or crossed adaptation refers to adaptation to stress arising from previous exposure to a different kind of stress. An example of specific adaptation is the development of antibodies to bacteria which protect the organism from subsequent infection by the same strain of bacteria but often afford no protection from bacteria of a different strain of the same species. An example of non-specific or crossed resistance discovered by Thatcher and Radike (65) is the increased resistance to sublethal doses of potassium which can be developed by repeated application of any one of a number of agents other than potassium. The adrenal cortex appears to be responsible for non-specific adaptation in this case because either Adrenal Cortical Extract (A.C.E.) or desocycorticosterone afforded protection of the same magnitude as non-specific adaptation.

The adrenal cortex does not appear to have any control over the mechanisms of specific adaptation such as antibody production or detoxification. Therefore, the acquisition of specific adaptation to

stress usually coincides with a decrease in adrenal cortical secretion from the elevated levels which characterize the non-specific reaction of the adrenals to stress. Langley and Clarke have (66) found that although there is at first a marked increase in the requirements for cortical steroid of adrenalectomized rats during exposure to low atmospheric pressure, continued exposure results in a decline in the steroid requirement to normal. This result has been confirmed by Darrow and Sarason (52). As has been noted previously, in Sayers "Type V" response of the adrenal cortex to stress, prolonged stress produces at first a decrease in sudanophilic material, cholesterol, and ascorbic acid in the gland followed by a return of the levels of these substances in the cortex to normal despite continued stress. Adaptation of adrenalectomized rats to the traumatic effect of rotating in a Noble-Collip drum occurs but its onset is slower than in intact animals (67). Possibly the earlier onset of adaptation in the intact animals is the result of non-specific resistance provided by the adrenal cortex, a resistance which disappears when the specific adaptation occurs at a later date as in adrenalectomized rats.

Selye (11, 68) has formulated the concept of the General Adaptation Syndrome to explain observations of his own and others concerning changes in resistance to stress in a period of prolonged stress and co-existent non-specific changes reflecting adrenal cortical function. He maintains that an animal treated for a long period of time with a daily sublethal dose of an alarming stimulus will exhibit a decrease in resistance during a period which he labels the "shock-phase". The resistance of the animal returns to normal during a "counter-shock phase"

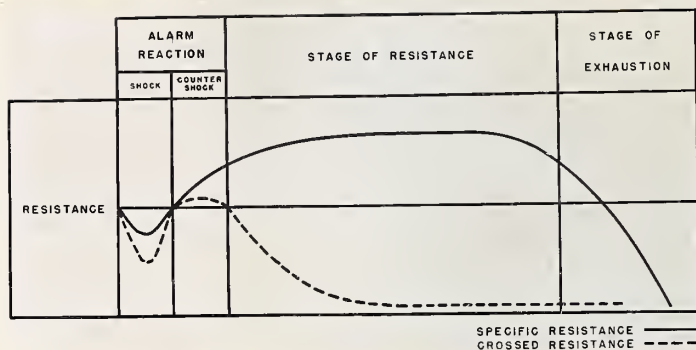


Figure 2. Selye's concept of the changes in resistance of an organism to super-added inquiry during a prolonged period of stress.

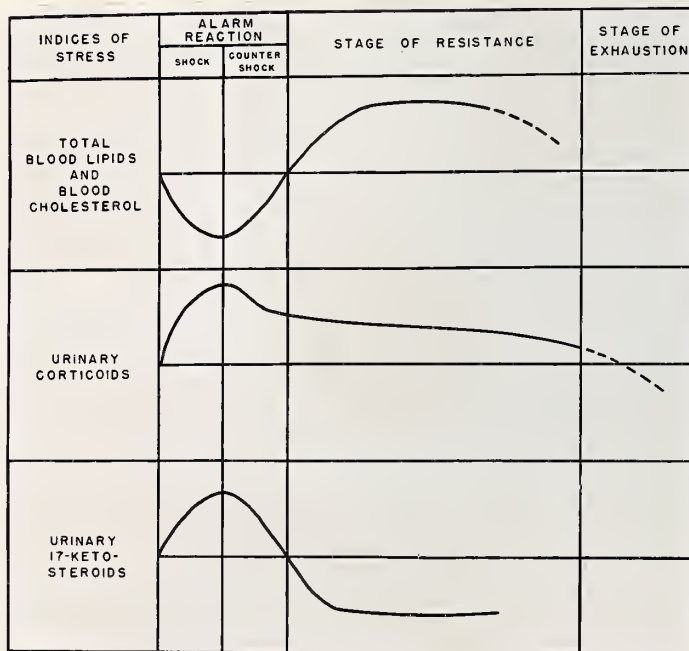


Figure 3. Alteration in total blood lipids and blood cholesterol, and in the urinary corticoid and 17-ketosteroid output in stress.

and rises above normal during the "resistance phase" (because of specific adaptation). Finally if the stimulus is applied for a long enough period, the acquired specific adaptation breaks down and the animal's resistance dwindles rapidly during final or "exhaustion phase" (see Fig. 2). The animal's resistance is determined by an additional minimum lethal dose. Apparently, according to Selye, the inherent "adaptive energy" of the organism falls short of the "adaptive work" required in maintaining specific adaptation.

At the same time as fluctuations are occurring in an animal's resistance to lengthy continuous application of sublethal doses of stress, Selye describes changes as occurring in the non-specific adaptation of the animals. During the shock-phase it decreases; it rises above normal in the counter-shock phase; during the resistance phase it drops gradually to a minimum level at which it stays until death occurs (see Fig. 2). Never, at any time, is the non-specific resistance as efficient as the specific adaptation as a homeostatic mechanism during stress.

According to Selye a number of morphological and physiological changes accompany the three main phases of the general adaptation syndrome. During the alarm phase, for example, tissue catabolism occurs with atrophy of the thymus and lymphoid tissue, lymphopenia, neutrophilia, ulcers of the gastro-intestinal tract and involution of the pancreas. The blood sugar is elevated during the shock phase of the alarm reaction and then drops to hypoglycemic levels in the countershock phase. The concentration of the blood non-protein nitrogen and potassium rise while the concentration of chloride in

the blood decreases during the alarm reaction. When the resistance phase begins, however, the morphological and biochemical changes of the alarm phase are restored to normal or in some cases are reversed so as to deviate to the opposite side of normal. For example, the hypochloremia of the alarm reaction is followed by a hyperchloremia in the resistance phase. During the exhaustion phase there is a return of the deviations from normal which characterizes the alarm phase. For an exact description of all the changes typifying general adaptation, the reader is referred to Selye's monograph "Stress" (68) which deals with the subject at some length.

Selye's statements concerning total serum cholesterol and the indices of adrenal cortical function are of interest, however. Selye claims that examination of available data shows "an initial decrease of cholesterol during the shock phase, followed by an increase during the countershock and early resistance phases" (69). He says that existing data are not sufficient to establish a trend during the late resistance phase and the exhaustion phase. Concerning the adrenal cortical secretory output, he says that it is greatest during the alarm reaction, continues above normal during the resistance phase and that it probably becomes deficient during the exhaustion phase (70). Selye attributes the depression of the 17-ketosteroid excretion, which was reported by Forbes, et al., (33) as occurring in the convalescent or resistance phase, to a possible shift in the pituitary hormone production with a decreased emphasis on production of gonadotrophic hormone and a greater emphasis the production of the hypothetical glucocorticotrophic and mineralocorticotrophic hormones (71). As a result the adrenal cortex

secretes less androgens and more gluco- and mineralocorticoids. Selye does not describe any behaviour for the eosinophil count beyond the well recognized eosinopenia of the alarm reaction phase of the General Adaptation Syndrome. All the fluctuations described in this paragraph are shown in Fig. 3.

B. Relationship of the Adrenal Cortex and the Serum Cholesterol Level.

I. The Effects of Stress on the Plasma Cholesterol Level.

Although some confusion exists in the literature concerning the exact effect of stress on the plasma cholesterol level of man and of animals, a few systematic studies provide considerable aid in resolving the contradictions that seemingly occur. Steiner and Turner (72) in 1940 found that, during the acute febrile stage of pneumonia, the plasma cholesterol level of human patients was depressed. Following this period, the plasma cholesterol rose to what were subsequently found to be hypercholesteremic levels for a considerable interval during convalescence. Finally the plasma cholesterol level subsided and became constant at a normal value characteristic of the patient under study. The average increase above normal in the series of patients of Turner and Steiner was 38% and the average duration of the hypercholesteremic phase was 52 days.

The finding of hypocholesteremia during the acute phase of stress has been described in human patients following surgery (73, 74), during pneumonia (72, 75), upper respiratory tract infection (75), other acute infections (76), and during the radiation treatment of malignancy (77). In 1920 Kipp (78) noted that hypocholesteremia

occurred in patients with pneumonia followed by a period of hypercholesteremia. According to him, the cholesterol level in the plasma was elevated during the period of convalescence when resolution of the exudate was happening. Stoesser (75), in 1938, noted that depression in the ester cholesterol titre of the plasma was chiefly responsible for the fall in the total plasma cholesterol during acute infection. According to Selye (69) several authors have reported a rise in serum cholesterol level caused by X-irradiation while others have described a fall in the cholesterol during the course of X-irradiation. Mattick and Buchwald (77) in 1927 found that X-irradiation produced a decline in the plasma cholesterol level of patients with carcinoma followed by a return of the plasma cholesterol level to pre-irradiation values in spite of continued radiation therapy. It is reasonable to suppose, therefore, that X-irradiation acts like other "stressor agents" in causing a period of hypocholesteremia followed by hypercholesteremia.

The experience derived from animal experiments appears to support the observations of Turner and Steiner and others on the pattern of the plasma cholesterol response to stress in humans. Exposure to low barometric pressure (53, 79), complete starvation (53), large doses of desoxycorticosterone acetate (53) and diethylstilbestrol (53) have been found to decrease the serum cholesterol level of rats. Repeated hemorrhaging has been reported to lower the plasma cholesterol levels of rabbits for two or three days (80) and then to produce a marked hypercholesteremia (80, 81) which eventually subsided. Also a single massive hemorrhage (81, 82) has been found to produce a sharp decline in the

ester cholesterol level content of the plasma. Ludewig, et al., (83, 84, 85) have reported rises in the plasma cholesterol level in response to burns and damage by vesicants in partially hepatectomized animals. Since the liver plays a very important role in the maintenance of the plasma cholesterol level, however, their results may merely reflect the liver damage in the experimental animals.

The biphasic pattern of the serum cholesterol response to stress coincides well with Selye's theory of the General Adaptation Syndrome. Selye (69) has described the hypocholesteremic phase of the cholesterol response as occurring throughout the alarm phase of the General Adaptation Syndrome and the hypercholesteremic phase as falling into the resistance phase (see fig. 3). The biphasic nature of the changes in cholesterol level in the plasma during stress certainly coincides well with Selye's theory of the changing bodily response to continued stress. When one attempts to correlate the variations in the serum cholesterol titre in stress with adrenal cortical function, however, one finds a dearth of experiments in which simultaneous observations were made of the indices of adrenal cortical activity and of the plasma cholesterol level. In 1945, Levin (53) reported that, during exposure to low atmospheric pressure a depletion of adrenal cortical stores of cholesterol occurred at the same time as a hypocholesteremia. Tepperman (79) has duplicated this result in rats exposed to a reduced atmospheric pressure equivalent to that found over an altitude of 15,000 feet. He found, however, that rats exposed to atmospheric pressures equivalent to lower altitudes demonstrated an increase rather than a decrease in the plasma cholesterol; the increase

in the plasma cholesterol occurred simultaneously with a depletion of the ester cholesterol content of the adrenal cortex. Generally in severe stress the period of hypocholesteremia during stress would appear to coincide roughly with the phase of depletion of adrenal cortical cholesterol. As the finding of Tepperman would tend to indicate, however, the two phenomena may not be directly related. Since depletion of the ester cholesterol content of the adrenal cortex has been found to be a good indication of cortical activity, the period of hypocholesteremia, therefore, is synchronous with increased adrenal cortical secretion in severe stress.

Sayers in his review on the subject of changes in the adrenal cholesterol content, states that a period of severe stress produces first depletion, and then, subsequent to the termination of the stress an accumulation of cholesterol in the adrenal cortex in greater than normal quantities. The period of accumulating cholesterol stores possibly coincides with the hypercholesteremic period which has often been observed in man and animals upon relief from severe stress. If such is the case, hypercholesterolemia may signal the restoration of adrenal cortical secretory activity to a normal level. Selye, however, interprets the period of hypercholesterolemia as happening at the time of the resistance phase of the General Adaptation Syndrome when the corticoid excretion is still elevated. Hence, by Selye's interpretation, hypercholesterolemia may indicate only an excess of adrenal cortical secretion over the body's needs.

The behaviour of the cholesterol level of the serum at the completion of convalescence contrasts markedly with its variability

during the period of stress and immediately thereafter. When patients had recovered from an attack of pneumonia, their cholesterol levels were found by Turner and Steiner (72) to become stabilized at a very constant value. The range of fluctuations in the normal subject was found to be much smaller than range of values in different subjects. Using the Sperry-Schoenheimer method, Sperry (86) had previously found that two or more determinations of serum cholesterol taken at intervals of up to twenty months were very close together in each of twenty-five adults. Turner and Steiner (87) found that even a diet high in cholesterol appeared to have little effect on the constitutional level of cholesterol in the serum. Morrison, et al., (88) have found, however, that marked fluctuations occurred in the serum cholesterol values in a series of fifty patients who had recently experienced coronary thrombosis and in consequence presumably had atherosclerosis. These fluctuations were uncovered by taking two determinations on each subject at intervals varying in length of time. Such a method of showing fluctuating cholesterol levels cannot reveal any pattern should one exist. Therefore, the possibility exists that the apparent fluctuations in the cholesterol level of the atherosclerotics were part of a pattern change in the cholesterol which occurs as one of the non-specific effects of any stress and that the atherosclerotic patients have their own constitutional non-fluctuating levels of cholesterol in the blood serum. Such a possibility is obviously important to the present study since all the patients in this study probably had some degree of atherosclerosis as the basis of their coronary heart disease.

II. Conn's Theory.

Conn, et al., in 1950, noted that ACTH produced a drop in the

serum cholesterol, particularly at the expense ester cholesterol fraction, in two normal people, in one patient with Cushing's disease, but not in a patient with Addison's disease (89). They attributed the effect of ACTH to the utilization by the adrenal, under the stimulus of the ACTH, of ester cholesterol from the blood plasma for the synthesis of adrenal cortical hormones. They postulated that the adrenal cortex was stimulated to such an extent by the ACTH that it produced hormones from the serum ester cholesterol faster than it could be esterified but not faster than cholesterol could be synthesized since the free cholesterol level remained unchanged. A lag of one day before ACTH produced its effect was explained on the basis that a day was required before the adrenal cortex exhausted its own supply of ester cholesterol and commenced utilizing that of the plasma.

The strongest piece of evidence supporting Conn's hypothesis is the experimental finding of Zaffaroni, et al., (10) that radioactive 17-hydroxycorticosterone can be isolated from surviving rat adrenal cortical slices perfused with fluid containing acetate or cholesterol tagged with ^{14}C atoms. A number of other facts fill in the background of Conn's hypothesis. In analysis of the adrenal cortex, it has been found that the adrenal cortex contained only minute quantities of adrenal cortical hormones (2) but large amounts of ester cholesterol (90) and ascorbic acid (91). This evidence strengthens the idea that the cortex manufactures its hormones as they are needed and secretes them directly into the blood stream without storing them for any length of time. The abundance of the ester cholesterol with the same cycloperhydrophenanthrene nucleus (see Fig. 1) as the cortical hormones suggests that it is the

precursor from which the hormones are manufactured. Sayers, Long and co-workers (4, 92) have demonstrated in the rat that ACTH and various kinds of stress produced a sudden, marked drop in the ester cholesterol and the ascorbic acid content of the rat's adrenal cortex. Rogers and Williams' pathological studies (50) on changes in the human adrenal gland in people dying of severe acute infection showed that these people have adrenal cortices depleted of cholesterol.

Rogers' and Williams' studies lend substance to the view that during severe stress the adrenal cortex might be exhausted of its store of ester cholesterol and might utilize plasma ester cholesterol instead. If the adrenal cortex uses plasma ester cholesterol after its own stores of ester cholesterol having been depleted during severe stress, it is theoretically possible that it might utilize the cholesterol faster than it could be esterified thus diminishing the ester cholesterol level in the plasma. If this supposition proves to be correct, the serum ester cholesterol level may afford an important means of gauging adrenal cortical activity during stressful situations.

The findings of Conn, et al., were at first specifically contradicted by those of Adlersberg, Schaeffer, and Drachman (93, 94). In a study of several patients, these workers noted than an increase in both total and ester cholesterol was produced both by ACTH and by cortisone therapy carried on for a long period of time. Subsequently, however, they found that ACTH caused a slight decline (95) (an average of -7%) in the first six to fifteen days of therapy and thereafter a moderate increase in the total and ester cholesterol levels (+11% and +13%, respectively). Cortisone, on the other hand, uniformly produced

a continuous gradual rise in the total and combined cholesterol levels. The results of the experiments of Adlersberg, et al., therefore, largely confirmed those of Conn, differing from Conn's results only in the degree of the drop in ester cholesterol that was produced. In addition, the clinical observations of Dustan, and others (96) on the effect of ACTH on two hypertensives support the findings of the above mentioned two authors.

The clinical observations to date on the effect of cortisone on the serum cholesterol levels are contradictory but in the main lend additional substance to Conn's theory. The study of Adlersberg, et al., (93, 97) comprises the largest and most pointed investigation of this aspect of cholesterol metabolism. As mentioned above, cortisone in doses of 25 to 200 mgms. per day produced a significant rise in serum cholesterol over a period of weeks. The rise occurred in both ester cholesterol and total cholesterol levels. It occurred in two patients on a fat-free, cholesterol-free diet (Kempner diet). If one modifies Conn's hypothesis somewhat this evidence concerning the effect of cortisone on the cholesterol metabolism can be fitted into the theory. The rise in cholesterol can be explained if one considers that the adrenal cortex utilizes ester cholesterol from the plasma at all times, not merely during stress. By decreasing the quantity of hormones that the adrenal cortex is required to produce, cortisone would cause the adrenal cortex to utilize less plasma ester cholesterol and the level in the plasma would rise. In this connection cortisone produces atrophy of the adrenal cortex (98) and apparently a marked diminishment of cortical function (99).

Clinical evidence against the theory of Conn is supplied by the observation of Perera, et al., (100, 101) and Dunstan, et al., (96) that in a small number of cases that cortisone produces a marked drop in the serum cholesterol level, a finding in direct contradiction to the work of Adlersberg and his co-workers. In addition earlier workers found that adrenal cortical extract produced a drop in the total serum cholesterol level (102). If cortical hormones produce a depression of the cholesterol level, then these hormones likely mediate the effect of ACTH which was observed by Conn in some way different than the one Conn hypothesized. Mason, et al., (25) in one patient observed a fall of free rather than ester cholesterol with ACTH therapy. Bloom and Pierce (103) found that neither ACTH nor cortisone affected the level of the special Sf 10-20 class of cholesterol-containing lipoproteins in the serum and that in eight of nine cases prolonged cortisone therapy failed to produce significant changes in the total serum cholesterol. It should be noted that Adlersberg's studies are based on more clinical material than those of the other groups. As a final point of clinical evidence, cases of Cushing's disease usually show no marked variations from normal in their serum content of total cholesterol (104), more often having hypercholesteremia than the hypocholesteremia which one would expect from Conn's theory.

In animal experiments Randles and Knudson (105) and others (106, 107), found that adrenalectomy produced no change in the serum cholesterol of rats. Pierce and Bloom (108) found that, in rabbits, ACTH and cortisone both produced an elevation in the serum cholesterol particularly the class of cholesterol-containing lipoproteins with a

floatation rate in the ultra-centrifuge of 40-400 Swedberg units (Sf). This evidence points to another explanation of the effect of cortisone and ACTH on lipid metabolism which will be dealt with in detail later on. Hoffmeyer (109) found that, in rabbits, transplantation of an additional adrenal gland into an animal caused a hypercholesteremia which was duplicated by adrenal cortical extract.

Summing up, the utilization of serum cholesterol by the adrenal cortex to synthesize corticoids during treatment with ACTH can be considered fairly well demonstrated. However, that this utilization of cholesterol effects any substantial decrease in the level of cholesterol in the plasma remains to be proven. Also remaining to be demonstrated conclusively is the exact effect of cortisone on the cholesterol metabolism and upon the serum content of cholesterol. At the present time there seems to be a considerable possibility that ACTH and cortisone may have antagonistic effects on the serum cholesterol level and that the effect produced by ACTH may be masked by the effect of the corticoids it stimulates the adrenal cortex to produce. Conn's theory, in short, remains to be substantiated.

III. Decreased Synthesis.

The liver exerts an important effect on cholesterol metabolism as indicated by many recent studies with radioactive isotopes. Gould (110) lately published a review article in which he gathered much of the evidence available to date. His conclusion based on work of his own as well as that of others was that the liver largely controls the serum cholesterol level. Though this conclusion has not been proven entirely, the evidence strongly suggests that it is actually the case. That the

liver synthesizes cholesterol was first suggested by clinical observation and animal experiments (111, 112, 113). Later on, the early suggestive evidence was corroborated by studies which showed that surviving liver slices in vitro utilized heavy water, deuterio acetate, and acetate labelled with C13 atoms in the buffer solution to produce cholesterol (114). Although cholesterol in the body is synthesized by many tissues, it has been shown in the dog that the liver is the only source of plasma cholesterol and that this cholesterol is free at first and is esterified after its release into the plasma (115). It can be seen, therefore, that changes in the liver synthesis of cholesterol caused by damage or by hormonal influence may produce the variations in blood cholesterol that are found during stress.

Pelner and Waldman (116) recently suggested that the anterior pituitary-adrenal cortical axis caused changes in the serum cholesterol by producing a fatty liver during stress. Considerable controversy has occurred in the literature as to whether or not the anterior pituitary with adrenal cortex or each gland separately produces a hormone which can cause a fatty liver. There is strong evidence that some hormone produced by the anterior pituitary-adrenal cortical axis is capable of producing a fatty liver. Anselmino and Hoffman (117) first discovered that some "principle" in a crude extract of the anterior pituitary produced fatty liver in rats into which the extract was injected. Subsequently ACTH (118, 119), growth hormone (119, 120, 121), and diabetogenic principle (122) with the synergism of cortical hormones (123, 124, 125) or without it have been claimed to be responsible for the production of the fatty liver. The most recent work (124, 125)

suggests that adrenal cortical steroids synergizes the fatty liver effect of some anterior pituitary hormone (both ACTH and growth hormone appear active). Whatever the hormonal mechanism by which the anterior pituitary-adrenal cortical axis acts, in stress this axis appears to cause a fatty liver (126).

The fatty liver results from the transfer of fats from the storage depots of the body and consequently the deposition of the fats in the liver (128, 129) in response to decreased carbohydrate utilization in the peripheral tissues (130, 131). According to Peters and Van Slyke, the effects of anterior pituitary extract (A.P.E.) proceed from carbohydrate starvation (32). Among the effects of the A.P.E. may be lipemia (133), ketosis (134, 135), and ketonuria (135) such as have been described in carbohydrate starvation. Theoretically, the hyperlipemia is not necessarily accompanied by hypercholesterolemia since it has been shown that phospholipids and cholesterol are associated with each other in the plasma (136, 137) but not with neutral fat or fatty acids (137, 138). The phospholipids and cholesterol tend to be combined in lipoprotein complexes containing very little neutral fat (137). In fact, however, hypercholesterolemia has been found to accompany the hyperlipemia of carbohydrate starvation (139, 140, 141). The possibility exists that the fatty liver of carbohydrate starvation is not closely similar to the A.P.E.-induced fatty liver and that the anterior pituitary in conjunction with the adrenal cortex produces hypocholesteremia associated with fatty liver. The evidence linking the A.P.E.-induced fatty liver and the fatty liver of carbohydrate starvation combined with fact that cholesterol is raised rather than lowered in carbohydrate starvation

tends to discount this theory. It is well known, however, that injury to the liver parenchyma producing a fatty liver causes simultaneously a marked drop in the plasma ester cholesterol in animals (108, 112) and man (111, 142, 143) alike.

The possibility even arises that, in cases of coronary occlusion, the liver parenchyma may be adversely affected by the physical effects of impaired heart action. Although myocardial infarction, per se, does not appear to have any effect on the liver circulation and consequently upon its function, according to the results of Kissane, et al., (144), congestive failure resulting from myocardial damage produces a marked impairment of liver activity (145, 146, 147). Although Kissane's study discounts the effect of shock and circulatory changes on the liver during acute myocardial infarction, too much reliance should not be placed on this work because the liver function test employed and all the present liver function tests provide only a relatively insensitive measurement of liver function. Furthermore, in the study of Kissane, et al., no mention is made of the length of time which elapsed after the myocardial infarction before the blood specimen for the liver function test was taken. Conceivably the tests were taken during the resistance phase of Selye when the bodily mechanisms had fully counteracted the deleterious systemic effects of the acute myocardial infarction.

In addition to the evidence provided by Kissane, et al., other evidence available supports indirectly the contention that depressed liver function is not responsible for any changes in serum cholesterol during stress. Three studies on rats have shown that regeneration of

liver tissue after partial hepatectomy in adrenalectomized (148, 149) or hypophysectomized (150) rats is slower than in rats in which the anterior pituitary and adrenals are intact. Furthermore in these experiments it was shown that desoxycorticosterone acetate and cortisone both restored the rate of liver regeneration of adrenalectomized animals to normal. These findings hardly seem compatible with any hypothesis indicating that adrenal cortical hormones during stress decrease liver function. However, further evidence must come to light before any conclusion can be made on the degree of derangement in the liver function occurring in severe stress and the effects on cholesterol metabolism.

Though there is considerable evidence to the effect that the anterior pituitary and adrenal cortex functioning together may cause a fatty liver during stress, there is no evidence at all that the fatty liver is akin to that found in injury to the liver parenchyma when a marked drop in serum ester cholesterol is found. The little evidence available tends rather to support the view that no depression of liver function occurs as of a result either of corticoid output or of the general systemic bad effects a severe stress such as myocardial infarction. No definite conclusions can be based on this evidence as yet because of its scanty nature.

METHOD:

A. Rationale.

The aim of this study was to provide a clear picture of the adrenal cortical response of the human organism to a severe stress and to relate to this any changes in the serum cholesterol which might occur. In order to achieve this aim, it was found necessary to study a small number of patients fairly intensively rather than to conduct a survey of a large number of patients. The reason for the limitation on the number of patients was the small number of patients with acute myocardial infarction available during the somewhat brief time during which the study could be carried on. Patients with acute myocardial infarction were chosen for the following reasons:

- (1) Some of these patients could be expected to suffer considerable stress and thus would present an opportunity to observe the changes in adrenal cortical activity and the serum cholesterol level in response to severe stress.
- (2) It was expected that some patients might die shortly after the myocardial infarction and thus might afford a chance to study the possible role of adrenal cortical exhaustion in causing death during severe stress.
- (3) Shortly after commencement of the study, it also became apparent that a study of the variations in the serum cholesterol level in patients with acute myocardial infarction and hence atherosclerosis might reflect some light on the problem of the genesis of atherosclerosis. Also after the study had been initiated, it was decided to include patients with acute coronary insufficiency in the study primarily

because of the difficulty of distinguishing immediately patients with acute coronary insufficiency from those with acute myocardial infarction. Since the former group of patients suffered a great deal of stress and since they were treated in much the same fashion as patients with acute myocardial infarction, no objection was seen to including them in this study.

In order to estimate as closely as possible the degree of stress to which a patient was subject, a daily record was kept of certain facts in the patient's objective and subjective state. Symptoms which were recorded were the presence or absence of pain and its intensity, if present, and the presence or absence of dyspnoea and also its intensity. On the objective side the patient's temperature (sublingual), pulse rate, and usually his blood pressure were followed daily. Any signs of embolism, edema or thoracic friction rub were noted. Finally a record was kept of such clinical laboratory investigations as were carried out. The laboratory investigations included the leukocyte count and differential blood count, the hematocrit, the erythrocyte sedimentation rate, the non-protein nitrogen, the fasting blood sugar and the electrocardiograms. The cephalin-cholesterol flocculation was carried out on five patients at the choice of the investigator.

The state of the patient's adrenal cortical response to stress was estimated from daily circulating eosinophil counts and from 17-ketosteroid and cortin assays performed on 24 hour urine collections obtained, if possible, every second day. The patient's total serum cholesterol was determined daily and in one patient a daily determination of the serum ester cholesterol level was carried out.

B. Clinical Material.

Seven patients with acute myocardial infarction and three patients with acute coronary insufficiency were studied. Of these patients, two were women, both with acute myocardial infarction. The patients ranged in age from 53 to 77 years. Two male patients (P.S. & D.B.) were mild diabetics and one woman patient (M.A.) was hypothyroid. Further details concerning these patients are summarized in Table I:

TABLE #I.

Clinical Material

<u>Hosp. No.</u>	<u>Initials</u>	<u>Sex</u>	<u>Age</u>	<u>Diagnosis</u>	<u>Probable Etiology</u>	<u>Previous Heart Disease</u>	<u>Hosp. Stay (Days)</u>	<u>Additional Treatment</u>
6091	M.A.	F.	53	Myocardial Infarction	Arteriosclerosis Hypothyroidism	Yes 1 mo.	32	Dessicated Thyroid
107133	D.B.	M	58	Myocardial Infarction	Arteriosclerosis Diabetes mellitus	No	34	Diabetic Diet
94483	T.L.	M	58	Myocardial Infarction	Arteriosclerosis Hypertension	Yes 5 yrs.	26	Low salt diet
72381	T.M.	M	59	Myocardial Infarction	Arteriosclerosis	Yes 5 yrs.	29	
107226	E.M.	M	55	Myocardial Infarction	Arteriosclerosis	No	35	
10065	M.R.	F	77	Myocardial Infarction	Arteriosclerosis	Yes 7 mos.	62+	
7638	P.S.	M	57	Myocardial Infarction	Arteriosclerosis Diabetes mellitus	No	43	Diabetic diet Insulin Cortisone 100 mg./dx4
17472	F.C.	M	61	Coronary Insufficiency	Arteriosclerosis	Yes <1 yr.	15	
56542	A.M.	M	51	Coronary Insufficiency	Arteriosclerosis	Yes 6 yrs.	24	
105948	S.S.	M	59	Coronary Insufficiency	Arteriosclerosis	No	30	

• Results Table 1 •

The following table summarizes the results of the analysis of the data collected during the fieldwork. The table is organized into four main sections: (1) General information, (2) Descriptive statistics, (3) Inferential statistics, and (4) Conclusions. The first section provides a brief overview of the study and the data. The second section presents the descriptive statistics for each variable. The third section shows the results of the inferential statistics, including the p-values and the confidence intervals. The fourth section contains the conclusions drawn from the analysis.

Table 1

Descriptive Statistics

Variable	Mean	Standard Deviation	Minimum	Maximum	Skewness	Kurtosis
Age	35.2	12.5	18	65	0.15	3.2
Gender	0.5	0.5	0	1	-0.1	3.0
Education	12.5	2.5	8	16	0.2	3.5
Income	1500	500	500	3000	0.3	3.8
Health	0.8	0.2	0	1	-0.2	3.1
Life Satisfaction	4.5	1.0	1	7	0.1	3.3
Stress	3.2	1.5	1	6	0.2	3.4
Depression	2.1	1.2	0	4	0.3	3.6
Loneliness	3.8	1.4	1	6	0.2	3.4
Quality of Life	5.2	1.1	3	7	0.1	3.2
Resilience	4.1	1.3	2	6	0.2	3.5
Optimism	4.8	1.0	3	6	0.1	3.3
Gratitude	5.5	1.2	4	7	0.1	3.2
Forgiveness	5.1	1.1	4	7	0.1	3.2
Compassion	5.3	1.2	4	7	0.1	3.2
Kindness	5.4	1.1	4	7	0.1	3.2
Generosity	5.6	1.2	4	7	0.1	3.2
Patience	5.7	1.1	4	7	0.1	3.2
Self-control	5.8	1.2	4	7	0.1	3.2
Emotional Stability	5.9	1.1	4	7	0.1	3.2
Psychological Well-being	6.0	1.2	4	7	0.1	3.2
Life Satisfaction (Control)	4.5	1.0	1	7	0.1	3.3
Stress (Control)	3.2	1.5	1	6	0.2	3.4
Depression (Control)	2.1	1.2	0	4	0.3	3.6
Loneliness (Control)	3.8	1.4	1	6	0.2	3.4
Quality of Life (Control)	5.2	1.1	3	7	0.1	3.2
Resilience (Control)	4.1	1.3	2	6	0.2	3.5
Optimism (Control)	4.8	1.0	3	6	0.1	3.3
Gratitude (Control)	5.5	1.2	4	7	0.1	3.2
Forgiveness (Control)	5.1	1.1	4	7	0.1	3.2
Compassion (Control)	5.3	1.2	4	7	0.1	3.2
Kindness (Control)	5.4	1.1	4	7	0.1	3.2
Generosity (Control)	5.6	1.2	4	7	0.1	3.2
Patience (Control)	5.7	1.1	4	7	0.1	3.2
Self-control (Control)	5.8	1.2	4	7	0.1	3.2
Emotional Stability (Control)	5.9	1.1	4	7	0.1	3.2
Psychological Well-being (Control)	6.0	1.2	4	7	0.1	3.2

C. Hospitalization and Treatment.

Since this study depended for clinical material on private patients, it was impossible to maintain uniform conditions of hospitalization. All the patients except one who had a previous history of diabetes (P.S.) were treated on the general medical wards of the hospital. P.S. was treated in the special metabolic ward. All the patients were treated with a basic regimen consisting of complete bed rest (at first), sedation, and dicoumarol sufficient to decrease the prothrombin time to 15% to 20% of normal. All the patients except the two diabetics and W.L. received the ordinary hospital diet. The diabetics (P.S. & D. E.) received special diabetic diets while W.L. received a low salt diet. P.S. was administered in addition insulin and during the auricular tachycardia, 100 mgms. of cortisone daily for four days and 50 mgms. one additional day. M.A. received dessicated thyroid for her hypothyroidism.

D. Clinical Observations.

The subjective condition of the patient was determined by direct questioning of the patient and by consulting the nursing notes. The temperature, pulse rate and blood pressure readings were obtained largely from the clinical chart of the patient. Physical signs were likewise obtained from the doctor's observations in the patient's clinical chart.

E. Collection of Samples.

(a) Blood.

All blood samples for the eosinophil counts, total serum and ester cholesterol determinations, erythrocyte sedimentation rates, and the cephalin-cholesterol flocculation tests were fasting blood samples obtained from the patient's arm veins before he or she had eaten breakfast.

All the various tests on these blood samples except the cholesterol determinations were carried out as soon as the blood was received in the laboratory. Since the cholesterol determinations require two days to perform and are started every second day, every second blood sample for cholesterol determination was held over in a refrigerator. In addition, for a period of about two weeks digitonin for the cholesterol determination was unobtainable and the blood serum samples collected during this period were kept frozen in a deep freeze locker until a determination could be carried out. It has been found by experience during routine laboratory work that freezing has no effect on the cholesterol level of samples of serum.

(b) Urine.

Twenty-four hour urine samples were collected from all patients except M.R. for 17-ketosteroid and cortin assays. Where possible, the urine samples were used immediately for these assays, but in many cases the urine samples were refrigerated as long as two days before the assays could be carried out. A period of refrigeration of as much as two or three days has been shown to have an insignificant effect on the results of the cortin and 17-ketosteroid determinations (151). In addition some samples were frozen and stored in a deep-freeze locker for a much longer period of time. As in the case of refrigeration for two or three days, the effect of freezing on the steroid assays of urine samples is negligible (151).

Creatinine determinations were carried out on all of the twenty-four hour urine specimens to ensure that the period of collection of the samples did not grossly exceed or fall short of the twenty-four hour

limit. In all cases except the ones noted in the results, twenty-four hour samples which showed a creatinine excretion out of line with other daily creatinine excretions of the same patient were discarded.

F. Technical Methods.

The daily cortin excretion was determined by the modification of the method of Daughaday, Jaffe, and Williams (21). Since Tompsett and Oastler (152) have stated that the trouble involved in carrying out the benzene-water partitions of the chloroform extract is not justified by the extra amount of information obtained by this procedure, it was omitted. Similarly, and for the same reason, no attempt was made to obtain the neutral ketonic fraction of the urinary steroids before assaying the 17-ketosteroids by the method of Holtorff and Koch (24). The staining method of Randolph (153) was used for the eosinophil counts. The total serum cholesterol and ester cholesterol levels were determined by a modification of the method of Sperry and Schoenheimer (154). The cephalin-cholesterol flocculation tests were done by the method of Hanger (155). The fasting blood sugar determinations were carried out by a micro-Schaffer-Hartmann Method (156).

OBSERVATIONS:

A. Variability of the Cholesterol Level.

In this study of the relationship of the serum cholesterol level to the degree of adrenal cortical functioning, perhaps the first and most striking feature to be observed from the data is the extreme variability of the serum cholesterol titre in these patients during cardiac distress. Though described as being relatively non-varying in normal individuals, the cholesterol level was found in this experiment to fluctuate considerably

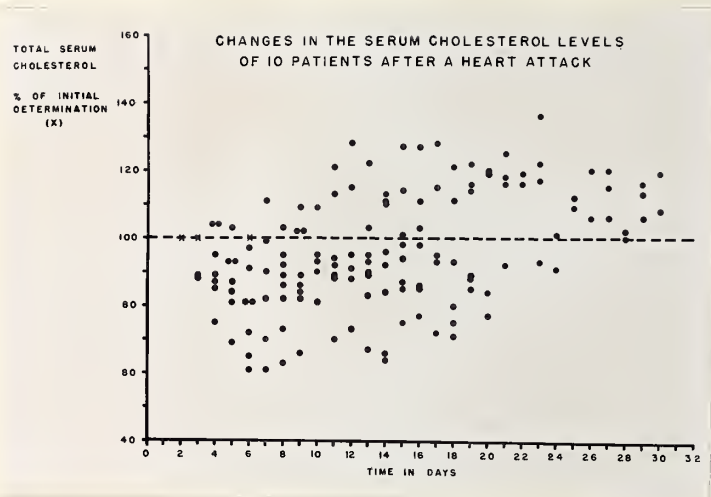


Figure 4. Changes in the serum cholesterol levels of 10 patients after a heart attack.

The daily cholesterol level of each patient is plotted as the percentage of the initial value.

as demonstrated by Table 2 in which the maximum absolute change in the cholesterol level of each individual is listed. During the period of observation, the greatest spread from the maximum to minimum cholesterol levels in one individual was 183 mgms.% in M.A. The least spread between maximum and minimum cholesterol levels recorded in an individual was 31 mgms.% in A.M. The average range between highest and lowest total serum cholesterol levels was 93.9 mgms.%. (See Table 2).

TABLE #2.

Variation in Total Serum Cholesterol Level.

Patient	Highest Level	Lowest Level	Difference
M.A.	445	262	183
F.C.	401	310	91
D.B.	235	188	47
W.L.	320	195	125
T.M.	330	277	103
A.M.	258	227	31
E.M.	299	220	77
M.R.	372	240	132
S.S.	308	267	41
P.S.	287	180	107
Average			94

Secondly, one may observe that the cholesterol level tended to alter in a systematic fashion following the myocardial infarction. Fig. 4 is a scatter diagram in which this tendency is illustrated by plotting the daily cholesterol levels of each of the individuals

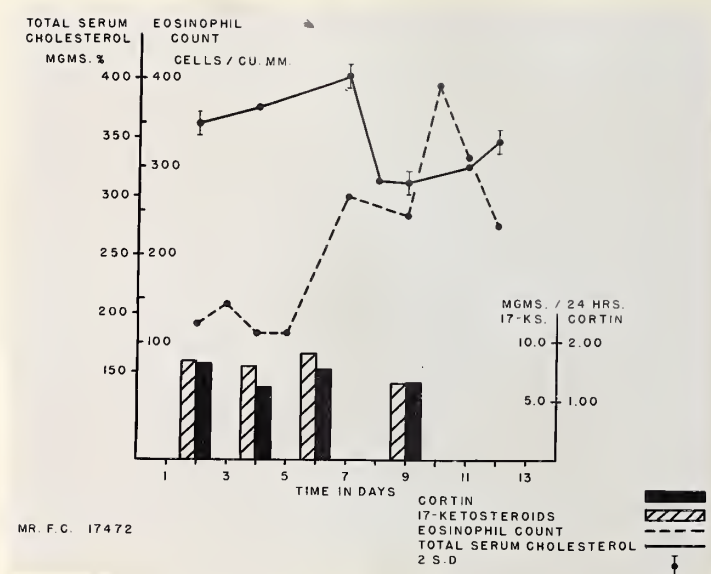


Figure 5. Data on Mr. F. C. #17472. This graph and the following nine graphs depict changes in the serum cholesterol, the urinary 17-ketosteroids and cortin, on the circulating blood eosinophils following a heart attack. The days are numbered from the onset of the attack.

throughout the observation period as percentage of that individual's initial cholesterol level. As may be seen, though the range of cholesterol levels is considerable on each day, there is a strong tendency for the cholesterol level to drop below the initial cholesterol level in the first days after the beginning of the cardiac disturbance and then a tendency for the cholesterol to return to an equal or higher level than the initial value when convalescence progresses.

B. Observations on the Relationship of the Total Serum Cholesterol Level to the Degree of Adrenal Cortical Activity during Stress.

Figs. 5 to 14 are graphs which were prepared depicting the changes in the total serum cholesterol and circulating eosinophil levels and in the cortin and 17-ketosteroid excretion in each of the ten individuals in the weeks following their heart attacks. A brief reference to these graphs will show that a considerable variability exists in the relationships of the various factors in different individuals. A short review of each individual case will next be presented, therefore, in order to provide a basis for the later general observations on the relationships of stress and adrenal cortical activity to stress. Excluded from these reviews are observations on the changes in 17-ketosteroid excretion which were, with but the few exceptions noted, so ill-defined as to contribute no information concerning the activity of the adrenal cortex.

F.C. (Fig. 5) - This patient entered hospital immediately after suffering an attack of acute coronary insufficiency. Despite the pain and emotional upset which he suffered, no elevation occurred in his cortin excretion in the first week after the attack or in the succeeding days. The only indication of an increase in adrenal cortical activity

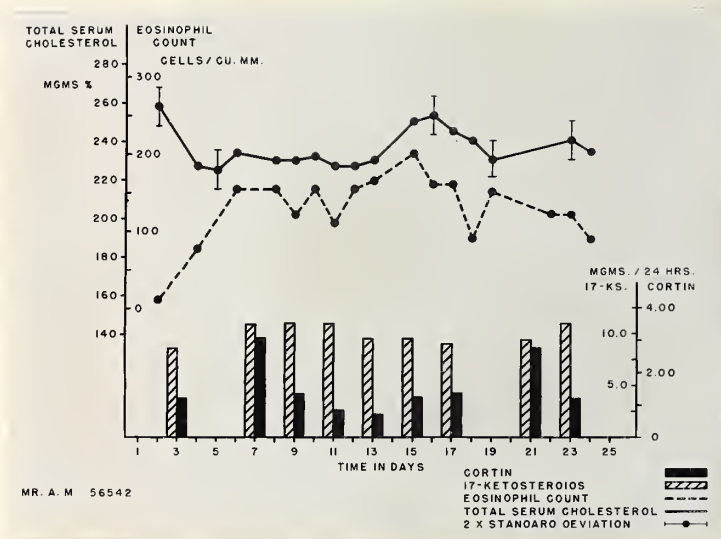


Figure 6. Data on Mr. A. M. #56542.

having occurred in response to stress was the depression in the number of circulating eosinophils in the first week as compared to the level of circulating eosinophils at the end of the period of convalescence which was observed. No decline occurred in cholesterol level from the initial value (taken on the second day) during the first week. Then at the end of the week the eosinophils rose sharply to a peak while the cholesterol dropped equally abruptly. Thus in this case, the drop in cholesterol level, if a response to stress, was delayed a week and coincided not with the period of eosinopenia but with the later period of eosinophilia during which the patient was apparently convalescing peacefully. The only abnormal sign at this time was a slight elevation in his blood pressure.

A.M. (Fig. 6) - A severe attack of coronary insufficiency resulted in this patient's being admitted immediately to hospital. The signs of the adrenal cortical response to this stress consisted of an initial circulating eosinophil level which was well below the fairly constant level which characterized the period of convalescence and also of an abnormal elevation of the cortin excretion which was first detected on the seventh hospital day. As indicated earlier, the highest normal excretion of cortin as determined by the method of Daughaday, et al., (21) is 1.50 mgms. for twenty-four hours. The serum cholesterol titre dropped from its initial level but did not reach its lowest level until a day before the eosinophil level had risen to its convalescent level. The serum cholesterol then remained depressed for about a week after both the cortin excretion and the eosinophil count had returned to what was apparently their normal constitutional level. Thus the

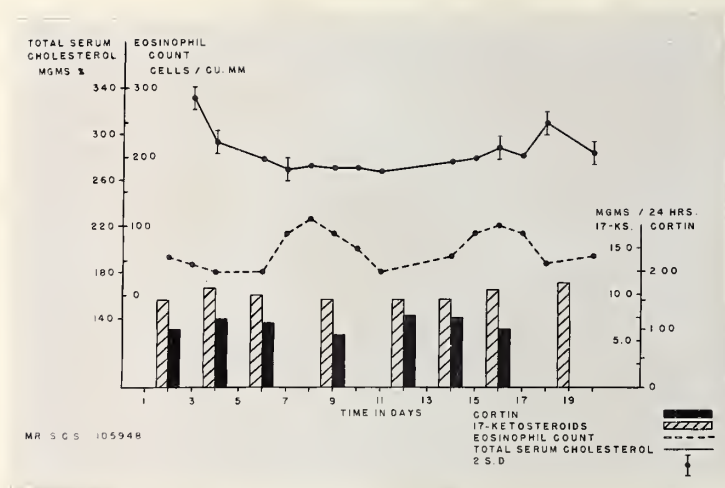


Figure 7. Data on Mr. S. S. #106958.

cholesterol titre of this individual remained low despite the disappearance of all signs of increased adrenal cortical activity. Finally just as the cholesterol level began to rise towards its initial level again, its progress was interrupted by another decline. This latter drop in cholesterol was apparently a response to an attack of angina which occurred the day previously. The maximum depression of cholesterol coincided roughly with an abnormal elevation of cortin excretion. The eosinophil count began to respond to the pain immediately, that is to say, it began to decrease a full day before the cholesterol level did so.

S.S. (Fig. 7) - This patient was admitted to hospital immediately after a very painful attack of coronary insufficiency. The only possible sign of increased secretory activity of the adrenal cortex was a fasting morning eosinophil count which, four days after the attack was less than a third of the peak value recorded during the patient's recovery. The serum cholesterol level declined from the initial level recorded two days after the attack but did not reach its point of maximum depression until after the eosinophil count had begun to increase. The serum cholesterol titre remained depressed throughout the rest of the period of observation, although it displayed some tendency to rise towards its initial level latterly. Its continued depression occurred despite any clear evidence of increased adrenal cortical secretion. Attacks of anginal pain did not produce any further lowering of the cholesterol level after its initial decline but possibly cut short a slight rise in the eosinophil count.

M.A. (Fig. 8) - An attack of myocardial infarction accompanied

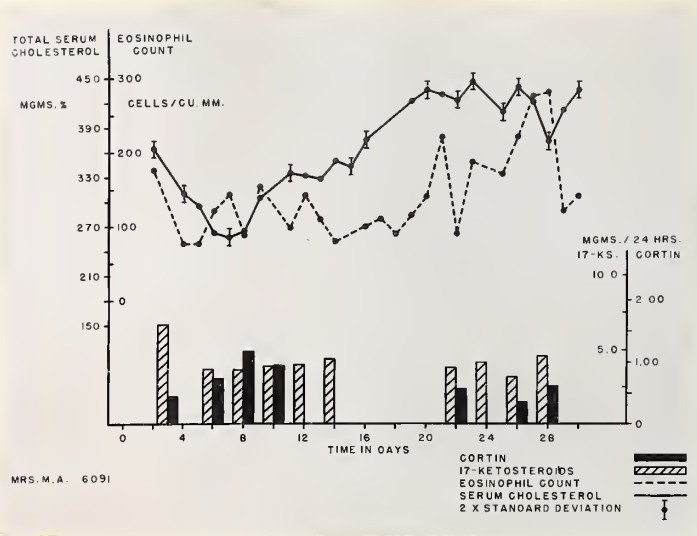


Figure 8. Data on Mrs. M. A. #6091.

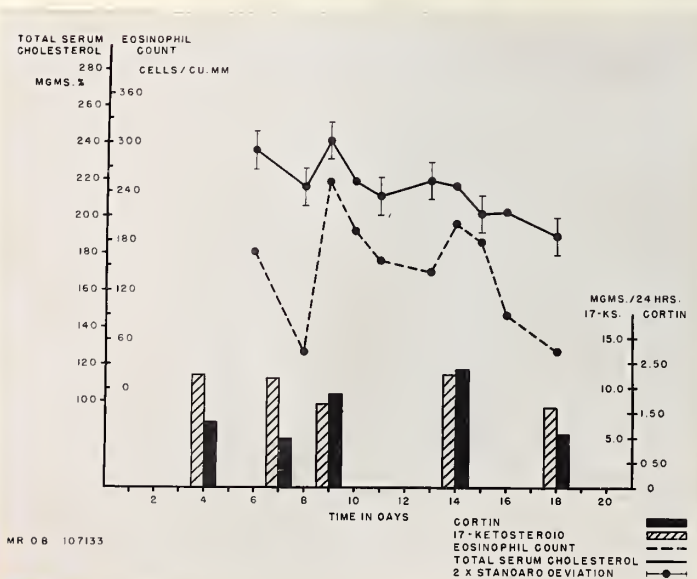


Figure 9. Data on Mr. D. B. #107133.

by severe distress brought this woman to hospital in a state of shock. The excretion of 17-ketosteroids three days after the attack was at peak level considerably higher than rate of excretion throughout the remainder of the period of observation. The number of circulating eosinophils fell from the second day level to a minimum value on the fourth day. This level was lower than all but a few values recorded later in the patient's recovery period. Two days after the eosinophil count began to rise, the cholesterol level, which had been declining from its initial value obtained on the second day, reached its lowest level. At the same time the cortin excretion reached a peak level which was considerably higher than convalescent values though still within normal limits. The serum cholesterol proceeded then to rise to levels well above its initial titre while the eosinophil count began to fluctuate reaching a peak while cholesterol level was elevated. An episode of angina on the twenty-sixth day after the infarction had no apparent reflection in the objective findings. A cholesterol determination taken over two months after discharge was higher than the lowest cholesterol level and lower than the most elevated level seen during the period of observation.

D.B. (Fig. 9) - This patient suffered a severe attack of myocardial infarction and was admitted to hospital. While in hospital, he was found to be diabetic and his diabetes was controlled by diet. The patient had a recurrence of pain on the fifth, sixth, and seventh days or just at the time observations on the serum cholesterol and circulating eosinophil levels were beginning. The cortin excretion was not high on the fourth day but began to increase on the ninth day

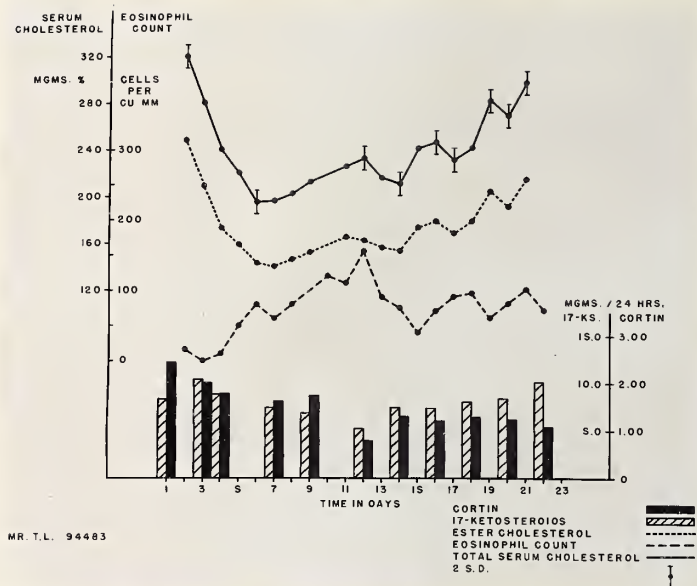


Figure 10. Data on Mr. W. T. L. #94483.
This patient is referred to alternately as T. L. and W. L. in the text.

by which time the eosinophils had demonstrated a sharp dip followed by a peak. The dip in eosinophils appears to have been a response to the second attack of pain. It was accompanied by only a slight dip in the cholesterol level from its initial value on the sixth day. After recovering, the cholesterol level began a downward trend in company with the eosinophil count. Though the cholesterol and circulating eosinophil levels continued on the downward trend until the end of the observation period, the cortin excretion returned to normal after reaching an abnormally high level on the fourteenth day.

W.L. (Fig. 10) - On the second day after this patient was brought to hospital suffering a massive myocardial infarction, the eosinophil count was found to be almost zero, a very low count compared to the values obtained in late convalescence. The cortin excretion on the first day was at a peak with a value about twice the daily excretion in the last week of the patient's hospital stay. The cholesterol level, on the other hand, though it dropped rapidly, reached a minimum level only after the eosinophil count had begun to climb and the cortin excretion to decrease. It then began to rise also and reached a secondary peak on the same day that the eosinophil count reached its peak level and the cortin excretion reached its minimum level. Thereafter both the number of circulating eosinophils and the serum cholesterol level began to fluctuate. At the same time, the cholesterol level continued in a general trend upwards towards the initial value recorded on the second day. Throughout the whole period of observation, the changes in the total serum cholesterol reflected faithfully the variations occurring in the serum ester cholesterol titre.

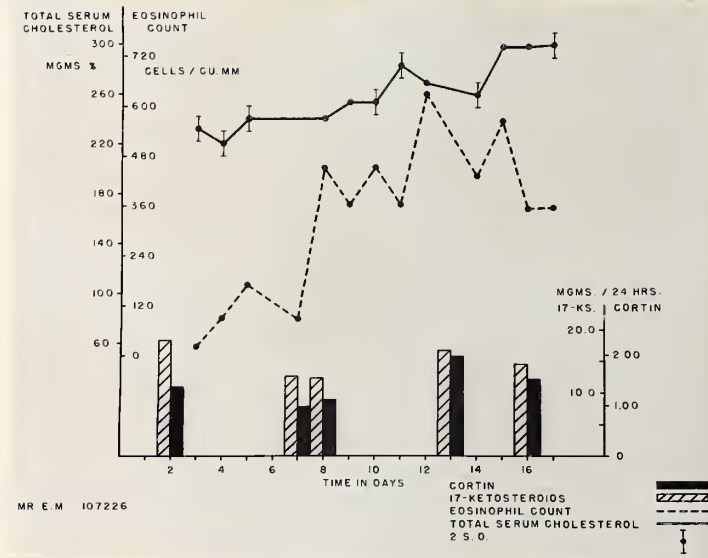


Figure 11. Data on Mr. E. M. #107226.

The cortin excretion levelled off at a value just about half the peak value as noted previously. It maintained a good inverse relationship to the circulating eosinophil level in all the period when observations were made.

E.M. (Fig. 11) - This man was brought into hospital following the development of severe chest pain caused by a relatively small myocardial infarction. No visible significant drop in cholesterol following the third day when cholesterol determinations were begun. For a week thereafter, the cholesterol level remained constant, afterwards beginning to rise. Meanwhile the number of circulating eosinophils also began to rise also from an initial level which was considerably lower than normal, judging by the eosinophil counts obtained at the end of the convalescent period observed. The cortin excretion was also at first at a low level compared to the values observed in the latter part of the recovery period and like the eosinophils hit a peak thirteen days after the attack and declined in the remaining period observed. The cortin excretion and the eosinophil level of this patient tended to vary directly with each other and therefore the picture of adrenal cortical activity is rather obscure. An attack of pain on the ninth day had no visible effect on the cholesterol and eosinophil levels but may have been responsible for the rise of cortin excretion which followed it.

T.M. (Fig. 12) - This subject suffered marked pain from a severe attack of myocardial infarction and was hospitalized immediately. Yet another attack of chest pain occurred two days after admission and this was probably responsible for the low level to which the circulating

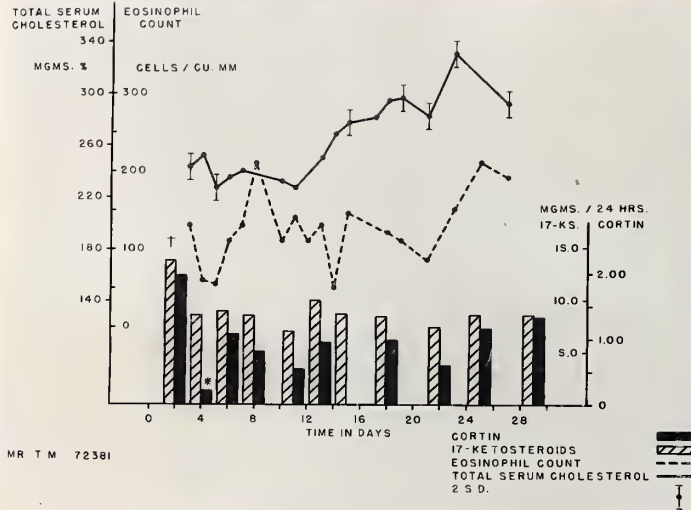


Figure 12. Data on Mr. T. M. #72381.

/ These 17-ketosteroid and cortin values probably exceed the actual levels, because the creatinine excretion for this 24 hour urine specimen is high.

* This cortin value is of dubious accuracy.

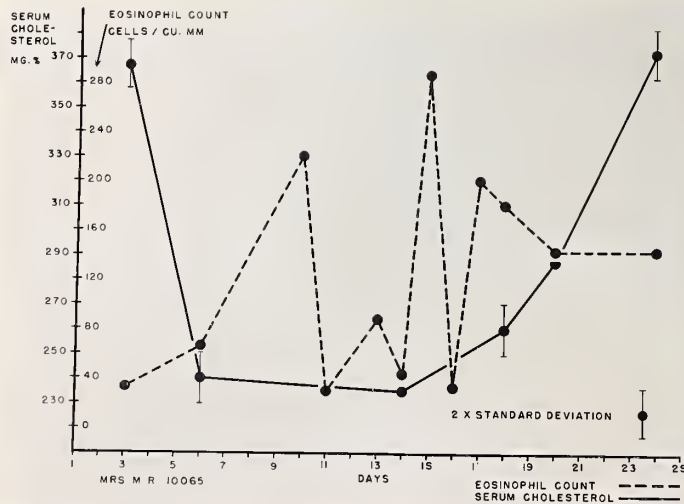


Figure 13. Data on Mrs. M. R. #10065.

eosinophils dropped on the fourth day. The high cortin excretion on the third day is somewhat misleading because the twenty-four hour creatinine excretion was also elevated above the value usually recorded in this patient. The cholesterol level in the serum remained fairly constant from the third to the twelfth day after which it began to rise. In the meanwhile the eosinophil count reached peak and then subsided and the cortin excretion hit a low two days later or about the same time as the cholesterol level in the serum began to increase. An attack of angina pectoris on the seventeenth day had no significant effect on the eosinophils, cortin or serum cholesterol. The cholesterol level reached a peak on or just after the twenty-sixth day; at about the same time the eosinophil count demonstrated a second peak.

M.R. (Fig. 13) - The serum cholesterol level dropped precipitously in this patient from the third to the fifth days after she developed a myocardial infarction. In this period, however, the circulating eosinophils were already recovering from an initial level (taken on the third day) which was very low compared to the eosinophil counts obtained at the end of the observed convalescent period. The eosinophil count began to fluctuate markedly thereafter presumably in response to the lesser afflictions which followed upon the myocardial infarction in this elderly patient. Throughout the period when the eosinophils were fluctuating the cholesterol remained well below its initial level. After the eosinophil count had developed a sharp peak and levelled out, however, the cholesterol level began to climb reaching the level of its initial value at the end of the observation period.

P.S. (Fig. 14) - This patient was hospitalized because of a

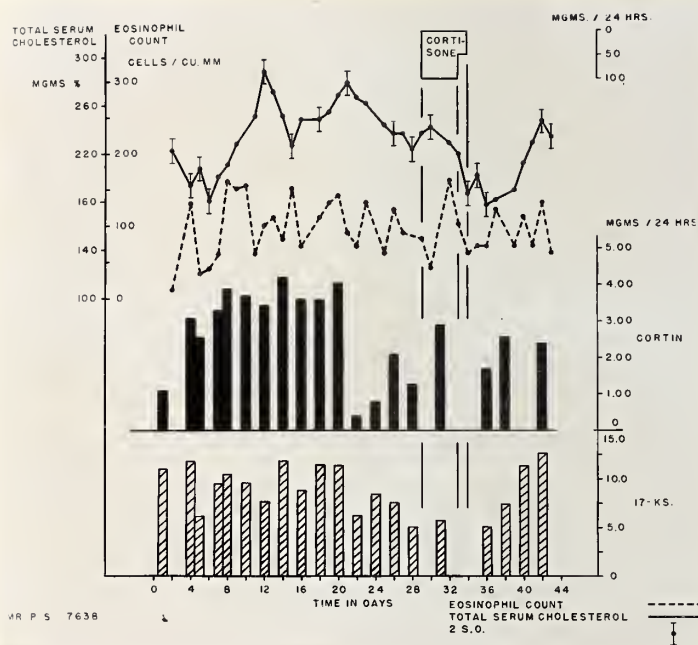


Figure 14. Data on Mr. P. S. #7638.

massive myocardial infarction. Twenty-one days after the initial heart attack, he developed auricular tachycardia accompanied by considerable pain and distress. Following termination of the tachycardia 14 days later, the patient recuperated satisfactorily.

The eosinophil count after the initial attack was low compared to late convalescent values. It began to rise while the serum cholesterol titre was still decreasing and then started fluctuating markedly without any apparent relation to stress or to the level of adrenal cortical secretion as indicated by the cortin output. The decline in cholesterol level was reversed before the cortin excretion reached its maximum level following the attack so that the cholesterol level and the cortin excretion were both climbing at the same time. The cholesterol then dipped again, though to a lesser extent, despite the fact the cortin remained abnormally elevated at a constant level. Following the onset of tachycardia on the twenty-first day, the cortin excretion immediately dropped drastically and then began to climb while the cholesterol level began a slow, steady decline which was only reversed significantly upon the termination of the tachycardia. The administration of cortisone in 100 mgm. daily dosage had no significant effect on the cholesterol level, although it apparently increased the cortin excretion twofold. A cholesterol determination carried out over two months later was about half way between the highest and lowest cholesterol levels observed.

C. Summary of Observations Encompassed in the Foregoing Review.

1. General Changes in the Serum Cholesterol Level and in the Indices of Adrenal Cortical Function with Stress.

(a) The total serum cholesterol dropped in the period just after the attack of myocardial infarction or ischemia. This drop took place in a period ranging up to a week after the attack. Then after an interval ranging from a day to two weeks it began to rise again towards the initial level recorded or to an even higher level. Only two patients, E.M. and T.M., failed to demonstrate the initial decline in the serum cholesterol titre. In these two individuals an abrupt, short-lived drop in cholesterol level could have taken place before the observations were begun three days after the attack. The cholesterol level in both of them rose in the weeks of recuperation in the same fashion as the other patients; this rise in cholesterol level was possibly a return towards the pre-stress cholesterol level. The only individual who failed to demonstrate the rise in serum cholesterol in the latter part of the observation period was D.B.

(b) The circulating eosinophil level was depressed in all individuals just after the attack of myocardial infarction or ischemia as compared to the later convalescent period. The only possible exception to this rule was S.S. in whom repeated attacks may have prevented the resting level of circulating eosinophils from being ascertained. In all individuals in whom unequivocal depression of both the eosinophil count and the serum cholesterol occurred following stress, the cholesterol response tended to be slower and to lag behind the eosinophil response. Another distinctive feature of the eosinophil response to stress was a peak in the eosinophil count which occurred after the eosinopenia. In six subjects (M.A., F.C., T.L., E.M., T.M., and M.R.) and to a lesser extent in two others (A.M. and P.S.), there was such a peak in eosinophil level, the eosinophilia falling sometime in the period when the cholesterol was rising after its period of

depression.

(c) The occurrence of an elevated cortin excretion in response to stress proved to be extremely variable in timing and degree. Two individuals, F.C. and S.S., showed no elevation of cortin excretion whatsoever. The largest group of individuals demonstrated a delayed but definite rise in adrenal corticoid excretion in response to stress. This group included M.A., A.M., E.M., P.S. and possibly D.B. and T.M. In the cases of M.A., A.M. and P.S. the elevated cortin excretion coincided roughly with the maximal depression in the total serum cholesterol level. One individual, T.L., showed an elevated corticoid excretion at about the time that the initial decline in cholesterol appeared to be starting.

(d) The 17-ketosteroid excretion showed no distinctive nor marked changes in response to stress except in the case of M.A., a woman, in whom an initial elevation of 17-ketosteroid excretion occurred. In T.L., a slight depression occurred throughout most of the patient's hospital stay.

2. Some Isolated Observations of Importance.

(a) A striking observation was the fact that in two individuals a decline in cholesterol level took place during stress despite a lack of clear evidence that the adrenal cortex was responding with increased secretion. When P.S. began to suffer tachycardia, the cortin excretion immediately dropped while the eosinophil count showed no striking change. Yet the serum cholesterol commenced a decline in its titre. In the case of S.S., the lowering in the serum cholesterol level took place following the attack of myocardial ischemia without any marked change in eosinophils and without any response in the cortin excretion whatsoever. Furthermore, in three other individuals, D.B., A.M. and T.M., the depression in

cholesterol level definitely outlasted the response of the cortin to stress. In the case of D.B., however, the eosinophil count was still low.

(b) In the one case in which serum ester cholesterol determinations were carried out, the changes in total serum cholesterol level were found to be reflections of variations in the ester cholesterol.

(c) In the five patients in whom the procedure was carried out cephalin-cholesterol flocculation tests in the period just after a heart attack were negative.

(d) The changes in the hematocrit readings of the subjects were small in comparison with magnitude of the alterations in the cholesterol levels.

DISCUSSION:

A. The Effect of Stress on the Serum Cholesterol Level.

The characteristic response of the level of the serum cholesterol to the stress of a heart attack was observed in this experiment to be a decline. When the findings of all the patients are taken together, the individual aberrations do not obscure this response. In seven out of ten subjects an initial decline in cholesterol level occurred which appeared to be a continuation of a previous downward trend of the serum cholesterol from its pre-stress level. In view of the fact that the great majority of the reports in the literature describe the cholesterol level as reacting in this fashion to various kinds of stress, there appears no obstacle to interpreting the decline in cholesterol from the initial recorded level after myocardial infarction or coronary insufficiency as part of a fall in serum cholesterol level from its pre-stress level. In the one individual (P.S.) in whom a cholesterol determination had been taken prior

the cardiac disease, the serum cholesterol one year before stress was considerably higher than the minimal level reached just after the attack.

A sharp decline in the cholesterol level of the individual (F.C.) did not occur until about a week had elapsed after his initial attack. This change in cholesterol appears to have been a response to stress also, however, because it accompanied a marked change in the eosinophil count. On the other hand, no increase in cortin excretion developed before, during and after the depression of the cholesterol titre in the serum so that there remains some doubt as to whether or not it actually represents a non-specific effect of stress.

This experiment does not provide sufficient grounds for discerning an actual period of hypercholesterolemia following the hypocholesteremic response of the cholesterol level to stress. No baselines in the form of observations of the serum cholesterol taken shortly before the occurrence of stress were available of course. It will be noted, however, that the peak cholesterol levels obtained in the two subjects M.A. and P.S. were higher than the serum cholesterol determinations taken over two months later when the cholesterol had time to become stabilized, assuming that the cholesterol level behaved in these individuals as it does following infectious diseases in otherwise normal individuals. In addition, the serum cholesterol determination carried out on P.S. a year before the heart attack was lower than his peak convalescent cholesterol level.

The question arises as to whether the changes in total serum cholesterol level actually reflected changes in the cholesterol metabolism

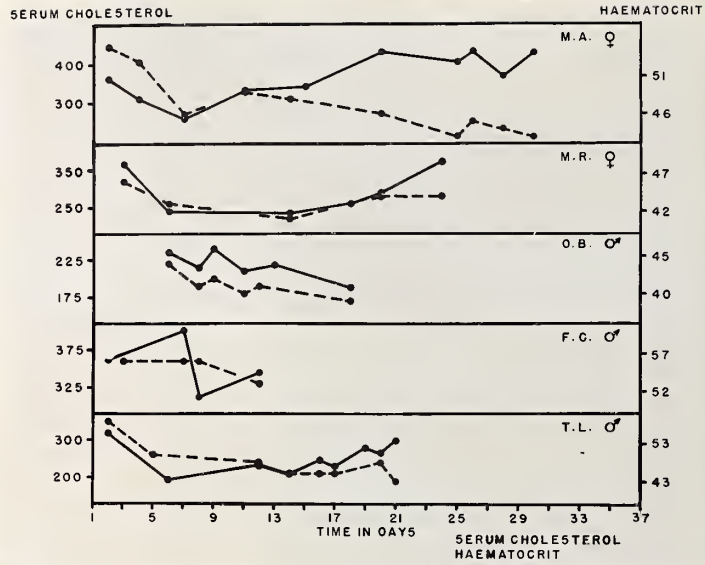


Figure 15. The relationship between the serum cholesterol and the hematocrit level of each of 10 patients following a heart attack.

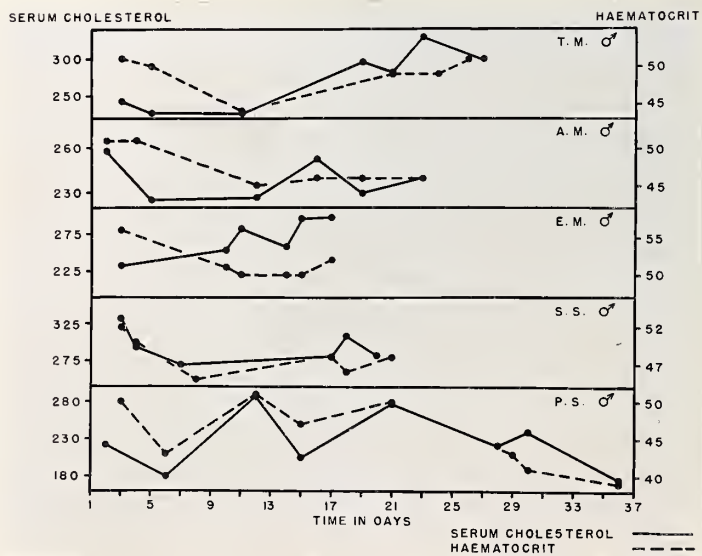


Figure 16. Continuation of Figure 15.

or simply varied with the fluctuations in plasma volume during stress. Man and Peters (157) in 1933 found that the plasma cholesterol level was affected by physiological transudation of plasma from the blood stream apparently because capillary walls of the vascular system are normally impermeable to cholesterol. One would naturally expect this finding to be correct since most of the cholesterol in the serum occurs in macromolecular form (136, 158). It follows therefore that the cholesterol level of the serum must be affected to a greater or lesser extent by the changes in plasma volume which accompany severe stress. In the case of P.S., the cholesterol level in the serum was found to vary usually in the same direction as the patient's hematocrit reading. The relationship of the hematocrit levels to the serum cholesterol in P.S. was typical of the relationship found in other patients, however, in that the changes in plasma water content were not sufficient to explain the major variations in the cholesterol during and after stress. Figs. 15 and 16 depict the changes in the hematocrit and cholesterol level which occurred concurrently in the various subjects.

Another possible explanation for the changes in the cholesterol found in the subjects of this experiment during stress is that the diets of the subjects were considerably curtailed in cholesterol, fat and caloric intake in the acute stage of the myocardial infarction or ischemia. On this basis one might attribute the changes in cholesterol level simply to changes in the patients' nutrition. Although no control of this factor was attempted during the experiment, evidence, both internal and external, is available which discounts the effect of diet on the cholesterol of the subjects during stress. To wit, in at least

three patients, W.L., A.M., and S.S., the depression of cholesterol in the blood serum occurring with stress long outlasted the acute phase of their illness when their nutrition would be expected to suffer. In addition, in the case of subject F.C., the depression in the patients cholesterol titre occurred not during the acute phase of systemic reaction when his appetite and consequently dietary intake would be expected to suffer but during the later period of apparent recuperation.

The evidence provided by other experiments favours the idea that the cholesterol level in the serum of an individual is relatively slightly affected by drastic changes in the dietary intake of cholesterol. An almost complete exclusion of fat from the diet appears necessary to produce a fairly rapid reduction in the serum cholesterol level (159, 160, 161, 162). Feeding a diet very high in fat and cholesterol will cause a slow but definite increase in the plasma cholesterol of humans (87, 163). The changes in dietary intake of these subjects appears therefore to have fallen far short of those required to effect even a moderate alteration in the serum cholesterol level. In the patient D.B., however, the downward trend of the serum cholesterol level throughout the period observation may be a reflection of the control of his diabetes by dietary means.

B. The Relationship of the Serum Cholesterol Level in Stress to Adrenal Cortical Activity.

Having examined the changes in the serum cholesterol alone during stress, consideration will next be given to the relationship of the changes in the cholesterol level to the variations in the indices of adrenal cortical function. There is some suggestion that the initial

decline in the cholesterol level following the onset of the heart attack was delayed for a day or two after the more immediate drop in the number of circulating eosinophils indicated the commencement of increased adrenal cortical activity. The cholesterol was definitely slower in achieving its maximum depression than was the eosinophil count. Following an attack of pain during the period of observation, A.M.'s eosinophil count responded with a decline a day before the serum cholesterol began to fall. F.C. exhibited a depression of cholesterol level which may be interpreted as a delayed response to an attack of coronary insufficiency to which the circulating eosinophils had responded a week earlier. These observations all constitute evidence for Conn's theory because Conn described a delay of about a day for the serum cholesterol level to become depressed after the beginning of treatment with ACTH (89). This delay he considered as the period required before the adrenal cortex was exhausted of ester cholesterol and utilization of the serum ester cholesterol as a precursor for hormones began. During this interlude, the eosinophil level could be expected to decline since the adrenal cortex was already secreting corticoids into the blood at an augmented rate. Thus upon the commencement of ACTH therapy or, equally, stress, a delay of about a day would be predicted from Conn's theory from the beginning of the drop in the eosinophil count to the beginning of the decrease in serum cholesterol titre.

The fact that the serum ester cholesterol level was the main cause of the variations in the total serum cholesterol in W.L. provides another bit of evidence in favour of Conn's hypothesis. Conn found that ACTH caused the variations in the total serum cholesterol by inducing

changes in the ester cholesterol level as did stress in the case of W.L. The support given by this evidence to Conn's theory is relatively minor because the changes in ester cholesterol level and consequently in the total serum cholesterol level might be caused by a decreased production of the ester cholesterol rather than by a utilization of it by the adrenal cortex.

In contrast to the feeble support which this study provides for Conn's hypothesis, the contradictions of the hypothesis which may be adduced from it are quite strong. The first contradiction is the fact that increased adrenal activity was not necessary to maintain the depression of the serum cholesterol level. In patient A.M., following his attack of coronary insufficiency, a considerable depression of the cholesterol level occurred before the cortin excretion became elevated and continued for about a week after the cortin excretion resumed what were apparently their normal levels. The same kind of occurrence happened in the case of T.M. D.B. showed a depression of cholesterol level after cortin excretion had dropped to low levels. In his case, however, the eosinophil count may have also been low indicating the possibility of some adrenal cortical activity. One must conclude that the prolongation of the depression of the serum cholesterol level after the disappearance of adrenal cortical emergency activity could only have been caused by a continuing effect of adrenal cortical hormones excreted in abundance during stress or directly by stress itself.

Not only was the serum cholesterol observed to remain low after adrenal corticoid secretory activity had subsided, but in the case of P.S. the serum cholesterol level was observed to begin dropping

following the onset of auricular tachycardia which reduced the cortin excretion drastically from a very high to a very low level. At the same time no striking fall in the number of circulating eosinophils occurred. On the surface, at least, it would appear that increased adrenal cortical activity is not even necessary for the commencement of the lowering in the serum cholesterol level in response to stress. One can explain away this obstacle to the acceptance of Conn's hypothesis on the basis that following the beginning of the auricular tachycardia the corticoid excretion was impaired giving a false impression of the degree of adrenal cortical secretory activity. There is no evidence to indicate that anything of the kind occurred. The creatinine excretion rate was normal shortly after the tachycardia began, although cortin remained depressed. Also there was no sudden excretion of abnormally high amounts of cortin following the termination of the tachycardia such as one might expect to occur in an attempt to remove the accumulated corticoids from the blood stream. However, it is possible that the corticoids were more efficiently utilized during the attack of tachycardia and for this reason less were excreted.

In subject P.S., also, the serum cholesterol began to rise to what might be interpreted as its pre-stress level despite the continued constant elevation of cortin excretion after the infarction. This fact offers no insuperable obstacle to the acceptance of Conn's theory however, since it is quite possible that homeostatic mechanisms compensated for the deficit of cholesterol in the serum produced by the adrenal's utilization of cholesterol in the synthesis of hormones. A homeostatic factor that might be involved in such a fashion is the production of cholesterol

in the liver. It does not seem impossible that the liver could maintain its functioning in this particular despite of the considerable systemic alteration produced in the organism by the myocardial infarction.

Possibly another objection to the validity of Conn's theory may be derived from the fact that the depression of eosinophils following myocardial infarction or ischemia seemed to disappear long before the depression in the cholesterol level in T.L., A.M., E.M., T.M., M.R., and to a lesser extent in M.A. and S.S. Outwardly this happening would suggest that the serum cholesterol level remained depressed long after the adrenal cortex had returned to its normal level of secretion. Thus the utilization of ester cholesterol in large enough amounts to explain the response of the serum cholesterol to systemic disturbance would be precluded if the suggestion is accurate. The question arises as to whether or not the eosinophils might become insensitive to the control of adrenal cortical hormones or might be produced in increased quantities so as to compensate for the effects of the hormones. Certainly the finding in P.S. that the eosinophil count rose to apparently normal levels, although cortin excretion remained elevated seems to point to such a conclusion. Ingle has noted that the effect of cortisone and similar hormones upon blood cells may be lost after a time or may be masked by a rise in the rate of the blood cells (164).

However, in other patients, the elevation in cortin excretion in response to the heart attack did not have a much longer duration than the eosinopenia. It seems possible, therefore, that the depression of the serum cholesterol titre following a stress actually lasted longer than the period of strenuous adrenal secretion in A.M. and T.M. and

possibly in others. Conn's theory provides no explanation for this situation as pointed out above.

A final piece of evidence against the hypothesis of Conn which was elicited from this study is the fact that the cortisone administered to P.S. during the period of his auricular tachycardia failed to evoke a significant rise in the serum cholesterol in spite the fact that it apparently induced a considerable increase in cortin excretion. Assuming the validity of Conn's theory, one should cause a marked rise in the serum cholesterol level by decreasing the quantity of corticoids which the adrenal cortex would be required to synthesize from serum ester cholesterol.

To summarize, therefore, the balance of the evidence supplied by this experiment tends to contradict the theory advanced by Conn, et al., that the adrenal cortex synthesizes adrenal hormones from ester cholesterol in the serum and thus lowers the titre of cholesterol in the serum.

Since the theory of Conn fails to find much confirmation in the evidence from this experiment, consideration will now be given to other possible mechanisms controlling the total serum cholesterol during and after stress. In the introduction the possibility has been entertained that the lowering of the serum cholesterol level is an effect of the adrenal cortical hormones poured out into the blood in relatively large quantities during stress. Mention was also made of the possibility that the depression in the cholesterol level might be a direct result of stress and not mediated at all by the adrenal cortex. Does the evidence of the experiment favour either one or the other of these possibilities? The answer is no. The changes in the cholesterol level

appeared to be related in time to the eosinopenia and subsequent eosinophilia elicited by stress. Yet at the same time, the depression in the serum cholesterol level appeared to have no quantitative relationship to either the amount or duration of adrenal cortical activity. This latter observation is in harmony with the experimental evidence of Adlersberg's group that cortisone therapy produces not a decline but an elevation in the cholesterol content of the serum.

On the other hand, all of the cephalin-cholesterol flocculation tests carried out on the five patients, M.A., D.B., A.M., T.M. and P.S. were negative indicating that the liver function was unaffected by the cardiac disorders. Since the liver probably controls the cholesterol level in the plasma more than any other organ, it appears that the effects of stress on the mechanisms normally controlling the cholesterol were not sufficient to explain the alterations in the serum cholesterol in stress. Nevertheless so little is understood about the way the liver acts in controlling the serum cholesterol level that too much importance should not be attached to a few cephalin-cholesterol tests. Also the cephalin-cholesterol flocculation depends upon some change in the albumin-globulin ratio (165); thus a sufficient interval may not have been allowed from the time of the heart attack to the time when the blood samples were taken for the protein balance to change.

The evidence from this experiment will next be reviewed in its relation to theories advanced by Selye about the relationships of the adrenal cortical activity and the serum cholesterol level in stress. As described earlier, Selye has postulated a biphasic response of the serum cholesterol in stress on the basis of his own experiments and of his

review of the literature on the subject. During the "alarm phase" of the General Adaptation Syndrome, Selye claims that there is a transitory depression of both the serum cholesterol and the circulating eosinophils. When the "alarm phase" gives way to the "resistance" phase, the serum cholesterol is considered by Selye to rise to levels considerably above the constitutional level in the unstressed organism. At the same time the depression of the eosinophil count ceases and beyond this point Selye does not carry his speculations.

The objection may be raised that the results of this experiment are not applicable to a discussion of the cholesterol level in the General Adaptation Syndrome because the G.A.S. results from a long continued stress while an attack of myocardial infarction is, on the contrary, relatively short in duration. The effects of myocardial infarction or ischemia in added strain on the myocardium are not so transitory however and must, in most cases, impose a residual handicap on the organism. In addition, the underlying condition of coronary arteriosclerosis which caused the myocardial disease is rarely materially affected by infarction and therefore, probably acts as a further source of stress to be overcome by the body's homeostatic mechanisms. Thus the condition of myocardial infarction is somewhat analogous to the state of prolonged stress which Selye states is the condition required for the General Adaptation Syndrome.

The fact that the period of serum cholesterol depression and the periods of low eosinophil counts during stress were found not to coincide has some bearing on Selye's theory. Invariably the depression in the number of circulating eosinophils appeared to terminate days or even weeks before the cholesterol level began to rise to what were

apparently normal levels. Thus either this experiment offers no guide to the consideration of the G.A.S. or Selye has misinterpreted the relationships of the serum cholesterol level and the circulating eosinophils during stress. Since all patients received bed rest, the impairment after the acute phase of the myocardial infarction or ischemia may have been eliminated. In this case, the picture in experiment may have only been that of the alarm phase without any element of the resistance phase entering into it. One would still expect, however, that the serum cholesterol to correlate closely with the eosinophil count but no such correlation occurs. Thus either the eosinophil count or the cholesterol level is not a good guide to the duration of the bodily reactions to stress which characterize the alarm phase of the G.A.S. or the theory is faulty in at least some of its time relationships. In fact, the cholesterol appears less satisfactory than the eosinophil count as an indicator of the alarm phase since maximum depression of the cholesterol level was delayed several days after the infarct in most of the ten cases studied. Also the depression of serum cholesterol appeared to linger on in all of the patients well into the period of convalescence when the patient gave no signs of acute bodily reaction to stress. This finding agrees well with that of Turner and Steiner (72) who observed that the period of depression of the cholesterol level during severe infection lasted for about twenty days.

As pointed out in the introduction, a large number of authors have described a depression of the total serum cholesterol level during and after severe disturbance to internal environment of the organism. The depression occurs in a wide variety of stressful circumstances so

that it may truly be considered non-specific. This experiment has demonstrated fairly well the same kind of reaction of the total serum cholesterol in patients with coronary arteriosclerosis who have suffered acute myocardial infarction or ischemia. It seems quite possible the variability in cholesterol level described by Steiner (166), Morrison, et al. (88), and Gertler, et al. (165), in arteriosclerotics without any outward manifestations of the disease may be more than just a direct specific effect of the arteriosclerotic process but rather may be an indirect non-specific stress response most likely caused by the arteriosclerosis. It is suggested, therefore, that though the subjects of these studies on the cholesterol level in relation to arteriosclerosis were free of symptoms from their basic condition that they may actually have been undergoing stress resulting from occult activity of the arteriosclerotic condition. The changes in the cholesterol levels of the subjects might have proved to be systematic rather than random fluctuations. Also these patients, when not subject to stress arising from their arteriosclerosis may have had the relatively stable constitutional total serum cholesterol levels which characterize normal people. This experiment points to the possibility that the regular determinations of the serum cholesterol levels of arteriosclerotic patients may be useful in determining the quiescence or activity of the arteriosclerotic disease if the patients are free of symptoms from disease.

Some comment should be made about the peculiar finding in the 17-ketosteroid excretion of M.A. This woman showed a definite peak in 17-ketosteroid excretion before the depression of the steroid output which characterized most of the period of the hospital stay. Such a

finding is distinctly atypical when it occurs in women because, according to Forbes, et al., (33) it is found in men only.

Summary and Conclusions:

- (1) A review is presented describing the theories about the relationship of the cholesterol level of the serum in humans to adrenal cortical secretory activity, particularly during stress. The importance of this relationship is outlined.
- (2) Some of the available experimental evidence on the changes in the serum cholesterol titre are presented.
- (3) A method is presented by which information can be obtained about the relationship of the serum cholesterol level to the degree of adrenal cortical activity during stress in a small series of clinical subjects.
- (4) Using the method indicated above, a study was carried out on a series of ten patients, three of whom had acute coronary insufficiency and the remaining seven had myocardial infarction.
- (5) The following conclusions were derived from the evidence of the study:
 - a. The total serum cholesterol level in the ten subjects was found to be extremely variable in the period just following an attack of acute myocardial infarction or ischemia.
 - b. The changes in the serum cholesterol in the arteriosclerotic patients followed a pattern. The changes probably consist of a sharp decline from the pre-stress level followed by a return to the pre-stress value during convalescence.
 - c. The relationship of the total serum cholesterol to adrenal cortical activity during stress is variable.

- d. Evidence was adduced that the ester cholesterol of the serum is not used by the adrenal cortex to produce hormones in sufficient quantities to cause changes in the cholesterol level of the serum during stress.
- e. The changes in the total serum cholesterol level in one patient were found to be a reflection of alterations in the ester cholesterol content of the patient's serum.
- f. The major changes in cholesterol level during severe stress are not caused by changes in the plasma volume.
- g. Five patients were found to have negative cephalin-cholesterol flocculation tests during the acute stage of myocardial disease.

A P P E N D I X

R O U G H D A T A

CLINICAL AND LABORATORY OBSERVATIONS

Mrs. M. A.

TABLE # 1

[illegible]

CLINICAL AND LABORATORY OBSERVATIONS

HOSPITAL NO 6091

Mrs. M. A.

[illegible]

CLINICAL AND LABORATORY OBSERVATIONS

HOSPITAL NO 174722

[illegible]

TABLE

Mr. D. B.

[illegible]

TABLE

CLINICAL AND LABORATORY OBSERVATIONS

TABLE #4

Mr. T. L.

- 5 -

HOSPITAL NO 94483

TESTS	Date Day	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	Apr 1
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Eos. Count			10	0	10	50	80	60	80		120	110	155	90	75	40	70	90	95
Total Serum Cholesterol			320	280	240	220	195	196	202	212		225	232	215	210	240	245	230	240
Ester Cholesterol			248	209	173	159	143	140	146	152		165	162	156	153	173	178	168	178
Creatinine	1.35			1.43	1.31			1.24		1.62			1.17		1.32		1.46		1.23
17 - K. A.	8.4			10.5	8.9			7.5		6.9			5.3		7.5		7.4		8.1
Cortin	2.46			2.03	1.80			1.64		1.76			0.8		1.31		1.22		1.30
Pain, Heart	+++	++	++																
Dyspnoea	+++	+																	
Temperature	98.8	98.6	101.0	100.6	100.4	98.8	99.6	98.6	98.6	98.2	98.2	98.4	98.4	98.4	98.2	98.4	98.6	98.0	98.0
Pulse	60	82	100	92	87	88	88	88	80	72	76	68	80	80	60	88	80	60	80
B.P.	200 104		120 89	115 89															
Sed. Rate		1	2	4	4				10		20	27	21	9	37	34	18	22	12

N.B. S.O.B. = Dyspnoea

Study Area				Data Collection Period			
Location				Date			
Area 1				Area 2			
Area 3				Area 4			
Area 5				Area 6			
Area 7				Area 8			
Area 9				Area 10			
Area 11				Area 12			
Area 13				Area 14			
Area 15				Area 16			
Area 17				Area 18			
Area 19				Area 20			
Area 21				Area 22			
Area 23				Area 24			
Area 25				Area 26			
Area 27				Area 28			
Area 29				Area 30			
Area 31				Area 32			
Area 33				Area 34			
Area 35				Area 36			
Area 37				Area 38			
Area 39				Area 40			
Area 41				Area 42			
Area 43				Area 44			
Area 45				Area 46			
Area 47				Area 48			
Area 49				Area 50			
Area 51				Area 52			
Area 53				Area 54			
Area 55				Area 56			
Area 57				Area 58			
Area 59				Area 60			
Area 61				Area 62			
Area 63				Area 64			
Area 65				Area 66			
Area 67				Area 68			
Area 69				Area 70			
Area 71				Area 72			
Area 73				Area 74			
Area 75				Area 76			
Area 77				Area 78			
Area 79				Area 80			
Area 81				Area 82			
Area 83				Area 84			
Area 85				Area 86			
Area 87				Area 88			
Area 89				Area 90			
Area 91				Area 92			
Area 93				Area 94			
Area 95				Area 96			
Area 97				Area 98			
Area 99				Area 100			

CLINICAL AND LABORATORY OBSERVATIONS

HOSPITAL NO94483

Mr. T. L.

[illegible]

CLINICAL AND LABORATORY OBSERVATIONS

HOSPITAL NO 72381

MR. T. M.

TABLE #5

[illegible]

CLINICAL AND LABORATORY OBSERVATIONS

188

Mr. T. M.

[illegible]

TABLE

TABLE # 6

HOSPITAL NO 56542

[illegible]

CLINICAL AND LABORATORY OBSERVATIONS

HOSPITAL NO 56542

[illegible]

CLINICAL AND LABORATORY OBSERVATIONS

HOSPITAL NO 107226

[illegible]

TABLE

- 12 -

TABLE #8

Mrs. M. R. (Admitted night of Dec 19)

HOSPITAL NO 10065

[illegible]

CLINICAL AND LABORATORY OBSERVATIONS

HOSPITAL 10065

Mrs. M. R.

[illegible]

TABLE

- 41 -

HOSPITAL NO 105948

TABLE # 9

[illegible]

CLINICAL AND LABORATORY OBSERVATIONS

- 15 -

HOSPITAL NO. 7638

[illegible]

CLINICAL AND LABORATORY OBSERVATIONS

- 16 -

HOSPITAL NO 7638

[illegible]

CLINICAL AND LABORATORY OBSERVATIONS

TABLE #10 (cont'd (3))

HOSPITAL 7638

[illegible]

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